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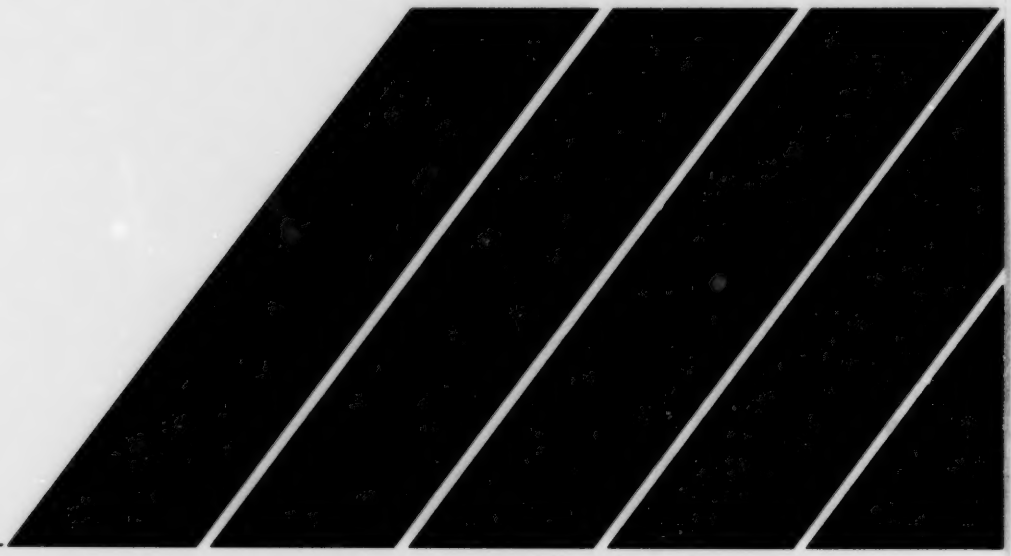
CHARACTERIZATION OF PRODUCTS DERIVED FROM THE PRETREATMENT OF BIOMASS



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CHARACTERIZATION OF PRODUCTS DERIVED FROM THE PRETREATMENT OF BIOMASS

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EXECUTIVE SUMMARY

The present progress report concerns the development of an analytical methodology to characterize and evaluate the efficiency (i.e., yields and products quality) of biomass fractionation and hydrolytic treatments.

Fractionation technologies are important to biomass conversion since their goal is to use each biomass constitutive polymer for specific applications compatible with their physical and chemical characteristics. Thermomechanical processes are inherent to biomass fractionation and pulping processes in general. These processes can be grouped in three generic variations:

TMV: this category relates to thermomechanical processes in which a "wetted or moist" feedstock (with or without addition of chemical agents) is treated with a saturated vapor. When steam is used, hydrolysis is induced. Mechanical effects can take place via sudden decompression through a blow-down valve. Steam-explosion processes (Iogen, Tigney and Stake are the canadian variations) are prototype TMV processes.

TMVA: this category involves thermomechanical processes in which the lignocellulosics are treated as a suspension (high water/wood ratio). Hydrolysis is induced by heating the suspension and can be enhanced by addition of sulfuric acid at low concentrations. Two main variations are to be considered: (a) the percolation approach (i.e. hydrothermolysis as initially described by Bobleter) and (b) continuous processing of the aqueous suspension in a suitable (CSTR or tubular) reactor. In our laboratory we use tubular reactors working in plug flow regimes with mechanical effects being induced by the use of on-line homogeneizing valves. Heating of the suspension can be carried out by indirect or direct (live) steam condensation.

TMS: thermomechanical solvolytic processes using the same features as TMVA but in the

presence of organic solvents. In our laboratory, ethylene glycol is used in a continuous mode at medium severity (200-250°C) for organosolv pulping process and at high severity (300-350°C) for cellulose depolymerization experiments.

All these thermomechanical processes lead to two main phases: a soluble fraction and a residue. Consequently, the development of the analytical techniques has been based on the separation of the two phases and their individual analysis. The objective is to present the yields of products in g/100g of initial lignocellulosics treated. Products of interest are the constitutive polymers derived from the original material and their physicochemical characteristics as a function of the treatment severity.

The first part of this report concerns the development of new analytical techniques applied to the treated materials. Determination of molecular weight distribution by size exclusion chromatography (SEC) has been applied to acetylated lignins and to cellulose tricarbanilates. Determination of crystallinity index and crystallite width by X-ray diffraction and ^{13}C nuclear magnetic resonance spectroscopy (NMR) of lignins in liquid state and of cellulose in solid state using cross polarization and magic angle spinning (CP/MAS) have also been developped.

The second part concerns the characterization of softwood treated by TMAV process with and without acid addition. Evaluation of the efficiency of the process as well as critical evaluation of the standard analytical methods for wood analysis are presented. Comparison between fractionation of hardwood and softwood is also discussed.

The last part of this report is addressed to the characterization of cellulosic residues obtained by depolymerization by acid hydrolysis and ethylene glycol solvolysis. A correction to the acid hydrolysis model is proposed and the mechanism of solvolysis depolymerization is presented.

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RÉSUMÉ

Le présent rapport porte sur la mise au point d'une méthode d'analyse pour caractériser et évaluer l'efficacité (c.-à-d. les rendements et la qualité des produits) du fractionnement de la biomasse et des traitements hydrolytiques.

Les techniques de fractionnement de la biomasse jouent un rôle important dans le domaine de la conversion de la biomasse puisque leur objectif est d'utiliser chaque polymère constitutif de la biomasse pour des applications spécifiques compatibles avec leurs caractéristiques physiques et chimiques. Pour le fractionnement de la biomasse et pour la réduction en pâte, on utilise des procédés thermomécaniques. Ces procédés peuvent être répartis en trois catégories :

TMV : il s'agit des procédés thermomécaniques qui consistent à traiter avec une vapeur saturée une matière de départ "mouillée ou humide" (avec ou sans addition de produits chimiques). Si l'on utilise de la vapeur, on induit l'hydrolyse. Les effets mécaniques peuvent se produire sous l'effet d'une détente grâce à une soupape de sécurité. Les procédés d'explosion à la vapeur (Iogen, Tigney et Stake en sont les variantes canadiennes) sont des prototypes de procédés TMV.

TMVA : cette catégorie désigne les procédés thermomécaniques dans lequels les produits lignocellulosiques sont traités comme une suspension (rapport

eau/bois élevé). On induit l'hydrolyse en chauffant la suspension et on peut améliorer l'hydrolyse en ajoutant de l'acide sulfurique en faibles concentrations. Il faut considérer deux variantes principales : a) celle qui fait appel à la percolation (c. -à-d. l'hydrothermolyse telle que décrite initialement par Bobleter) et b) celle qui consiste à traiter en continu la suspension aqueuse dans un réacteur approprié (CSTR ou tubulaire). Dans notre laboratoire, nous utilisons des réacteurs tubulaires à écoulement piston, les effets mécaniques étant induits grâce à des soupapes homogénéisantes en ligne. Pour chauffer la suspension, on peut faire appel à la condensation de vapeur indirecte ou directe (vapeur vive).

TMS : il s'agit des procédés solvolytiques thermomécaniques présentant les mêmes caractéristiques que les procédés TMVA, mais qui sont appliqués en présence de solvants organiques. Dans notre laboratoire, on utilise de l'éthylèneglycol en mode continu à température moyenne (200 - 250 °C) pour les procédés de réduction en pâte de type organosolv et à température élevée (300 - 350 °C) dans les expériences de dépolymérisation de la cellulose.

Tous ces procédés thermomécaniques produisent deux phases principales : une fraction soluble et un résidu. C'est pourquoi la mise au point des techniques d'analyse a été basée sur la séparation des deux phases et sur leur analyse indiviuelle. L'objectif est de présenter le

rendement de produits en g/100 g de matière lignocellulosique traitée initialement. Ce qui nous intéresse, ce sont les polymères constitutifs provenant de la matière d'origine et leurs caractéristiques physico-chimiques en fonction de la température de traitement.

La première partie du rapport porte sur la mise au point de nouvelles techniques d'analyse appliquées aux matières traitées. On a déterminé la répartition des masses moléculaires par chromatographie d'exclusion (SEC) dans le cas des lignines acétylées et des tricarbanilates de cellulose. On a également mis au point des méthodes pour déterminer l'indice de cristallinité et la largeur des cristaux par diffractométrie X et par spectroscopie de résonance magnétique nucléaire de ^{13}C (RMN) des lignines à l'état liquide et de la cellulose à l'état solide en faisant appel à la polarisation croisée et à la rotation de l'angle magique (CP/MAS).

La deuxième partie concerne la caractérisation du bois de confières traités par TMAV avec et sans addition d'acide. On présente une évaluation de l'efficacité du procédé ainsi qu'une évaluation critique des méthodes standard d'analyse du bois. On compare également le fractionnement du bois de feuillus et du bois de conifères.

La dernière partie du rapport porte sur la caractérisation des résidus de cellulose obtenus par dépolymérisation avec une hydrolyse acide et une solvolyse à l'éthylèneglycol. On propose un modèle d'hydrolyse acide corrigé et on présente le mécanisme de la dépolymérisation par solvolyse.

1: INTRODUCTION

The Biomass Thermochemical Conversion Research Group of the University of Sherbrooke, with E. Chornet and R.P. Overend as leaders, has as major objective the controlled fractionation of wood, peat or agricultural wastes into their constitutive polymers as primary conversion fractions for further processing via acid/base depolymerization, chemical modification (grafting), selective hydrogenation, enzymatic hydrolysis, etc. The process development units (PDU) available at the UdeS are the following[1]:

- UdeS-F process: this is a continuous aqueous/steam fractionation unit with shear devices that increase defibration and defibrillation of the lignocellulosics[1];
- UdeS-H process: this process is based on the unit described above but with automated addition of acid in the reactor to accomplish the hydrolysis of biomass[2];
- UdeS-S process: this is a continuous unit for biomass solvolysis using organic solvents from room to high temperature[3,4];
- MVC unit: or mini-vapo-cracker, a discontinuous, "steam explosion" 2 L vessel for a variety of tests with either steam or organic vapours. Small chips (2-4 mm), shavings or sawdust can be used in this unit;
- Stake pilot plant: this steam explosion unit can treat up to 4 t/h of either chipped wood, shavings or sawdust.

The intensive development of the analytical section of the group began in 1983[5-7] and has been pursued until the present contract with the following objectives:

- development and implementation of analytical methodologies for the characterization of biomass;

---

1 All the PDU's named UdeS use powdered wood suspension feedstock (10% w/w) and operate at a suspension flow rate of about 1 kg/min.

- evaluation and improvement of the current methods in relation to their reliability (or lack of it) when dealing with high severity treated biomass samples;
- development of analytical tools for characterization of the constitutive polymers, modified either structurally or chemically;
- development of an analytical strategy which gives the complete mass balance of the different products derived from the initial biomass.

2: OBJECTIVES

The general objectives of the present contract with the Bioenergy Development Program are the following:

- Assure the development and the implementation of an analytical methodology for the analysis of biomass treated by different thermochemical processes;
- Propose new methodologies for treated biomass and the fractions containing their constitutive polymers in order to acquire information about their structures, molecular weight distribution, chemical modification, and other properties;
- Integrate these analytical methods in a general procedure which will give insight on the samples as a function of the severity of the treatment;
- Relate our methodology to the procedure used by other laboratories in order to help to establish an unified analytical procedure.

The specific objectives of the present contract with the Bioenergy Development Program are the following:

- generalize the analytical methodology proposed for hardwood[7] to softwood treated by aqueous/steam fractionation process with and without use of acid as catalyst;
- pursue the characterization of cellulosic residues obtained from partial hydrolysis in processes using dilute acid solutions;
- pursue the characterization of cellulosic residues obtained from depolymerization by solvolysis in ethylene glycol;
- characterization of lignin obtained from wood solvolysis in ethylene glycol and subsequently treated by alkaline or fractionated by organic solvents.

3: ANALYTICAL METHODOLOGY

The analytical procedure used in Sherbrooke for the characterization of soluble and residual fractions obtained from biomass fractionation processes has been described previously[6,7]. Figure 3.1 presents our analytical strategy for the characterization of the different fractions obtained from aqueous/steam fractionation and hydrolysis of wood.

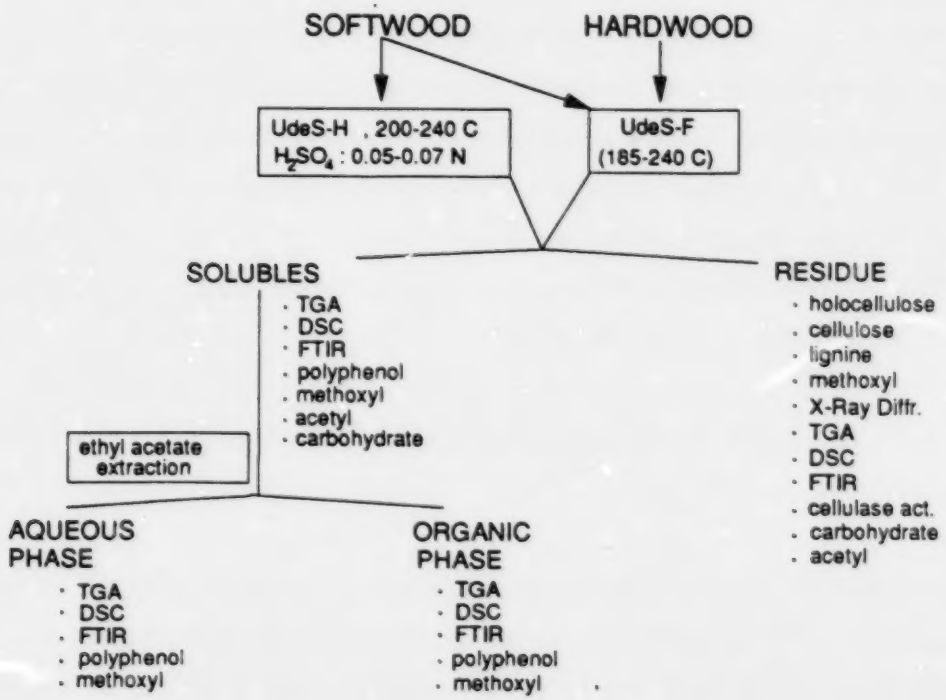

Figure 3.1: Aqueous/steam fractionation experiments and the analytical procedure used for the characterization of the different fractions

Three new analytical methods have been implemented in the analytical section of our research group during the present contract. The procedure for the determination of the molecular

weight distribution of cellulose and lignin by size exclusion chromatography (SEC) in organic solvent has been successfully developped. The determination of crystallinity index and crystallite width of wood and cellulose has been done by X-ray diffraction analysis. Finally, the determination of chemical functionalities of lignin and cellulose has been carried out by ^{13}C NMR in liquid state or in solid state using CP/MAS technique. These methods are described in the following subsections.

3.1 Determination of molecular weight distribution by size exclusion chromatography (SEC)

In the case of homopolymers like cellulose, a polydisperse sample can be represented by a distribution which can be uni-, bi- or multimodal and can be described by specific average values: $\overline{M}_N$: number average molecular weight; $\overline{M}_W$: weight average molecular weight; $\overline{M}_V$: viscosity average molecular weight; $\overline{M}_Z$: Z average molecular weight. These specific values are described by the following equations:

$$\overline{M}_N = \sum_i x_i M_i = \frac{\sum_i (n_i M_i)}{\sum_i n_i} \qquad \overline{M}_W = \sum_i w_i M_i = \frac{\sum_i (n_i M_i^2)}{\sum_i (n_i M_i)}$$

$$\overline{M}_V = \left(\sum_i w_i M_i^{\alpha}\right)^{1/\alpha} = \left(\frac{\sum_i n_i M_i^{1+\alpha}}{\sum_i n_i M_i}\right)^{1/\alpha} \qquad \overline{M}_Z = \frac{\sum n_i M_i^3}{\sum n_i M_i^2}$$

where:
- n_i is the number of molecules of molecular weight M_i;
- x_i is the molar fraction of polymer with mass M_i;
- w_i is the mass fraction of polymer with mass M_i;
- α is an experimental constant for a particular polymer-solvent system.

Polydispersity ratios can also be calculated and give indication of the dispersity of the

molecular weight distribution:

$$P = \frac{\overline{M}_W}{\overline{M}_N} \qquad P' = \frac{\overline{M}_Z}{\overline{M}_W}$$

P and P' are identical in a logarithmic distribution while P is greater than P' for an exponential molecular weight distribution. A value of 2 for P is typical of a Schulz-Flory distribution which is encountered in a random mechanism of depolymerization.

3.1.1 Size exclusion chromatography of acetylated lignins

The analysis of acetylated lignins obtained from different treatments by size exclusion chromatography was performed with a Varian 5000 liquid chromatograph connected to an on-line computer (NEC APC IV) via a Waters Maxima 820 data system. Two stainless steel columns connected in series were used (300 x 7.5 mm) packed with PLgel spherical particules (Polymer Laboratories) of particule size 5-10 µm, and pore size of 50 and 500 Å respectively. Detection was made with a UV monitor at 254 nm. A constant flow rate through the system was maintained by a single-piston reciprocating pump. The following operating conditions were utilized: eluent, tetrahydrofuran (continuously bubbled with N_2 to prevent the formation of peroxides); flow rate, 1 mL/min; pressure, 7.0 MPa; temperature, 20-25°C; injection volume, 10 µL; sample concentration, 1mg/mL.

The calibration curve was obtained using monodisperse polystyrene compounds of known molecular weight as reference substances. A third-order polynomial of the form $y = ax^3 + bx^2 + cx + d$ best describes the relation between the log of the actual molecular weights of polystyrene standards (y) and their retention volumes (x). The following coefficients were calculated: a = -0.00714, b = 0.3253, c = -5.149, d = 30.69. These gave a fit with a multiple regression coefficient of r^2 = 0.998.

3.1.2 Size exclusion chromatography of cellulose tricarbanilates

The properties of tricarbanilates for the study of cellulose and starch have been discovered by Burchard and Husemann[8]. The advantage over the nitration procedure is a complete substitution of the hydroxyl groups in one step without degradation of the cellulosic chains[9,10]. The structure of cellulose tricarbanilate is presented in Figure 3.2.

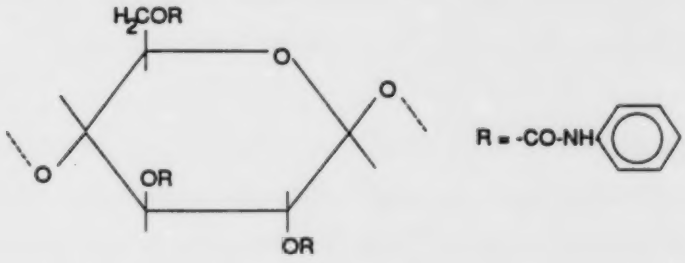

Figure 3.2: Structure of cellulose tricarbanilate (CTC)

The procedure of carbanilation is the following: About 50 mg of freeze-dried cellulose are suspended in 20 mL of pyridine and 2 mL of phenylisocyanate are added. After 48 hours of incubation at 80°C under agitation, the reaction is stopped by addition of 2 mL of methanol and the CTC is precipitated by pouring the solution in 150 mL of a 30% methanol solution. After centrifugation, the precipitate is washed with the methanol solution followed by water and freeze-dried. The yield of carbanilation is greater than 95%.

In the case of the determination of the molecular weight distribution of cellulose we have decided to use the universal calibration as proposed by Benoit *et al.*[11,12]. This calibration uses the hydrodymamic volume function ($[\eta] \cdot M$) instead of the molecular weight for the making of the calibration curve. From this function we are able to convert the molecular weight of the polystyrene used for the calibration curve to cellulose tricarbanilate molecular weight by the following equation:

$$M_{CTC} = \left[\frac{K_{PS} \quad M_{PS}^{(\alpha+1)_{PS}}}{K_{CTC}} \right]^{\frac{1}{(\alpha+1)_{CTC}}}$$

In order to resolve this equation, we need to know the coeffficients K and α for both polystyrene and CTC in THF. We have used the mean of these coefficients proposed in the literature[13-16]: $\alpha_{PS} = 0.699$, $K_{PS} = 0.0158$, $\alpha_{CTC} = 0.857$ et $K_{CTC} = 0.0040$.

A third-order polynomial of the form $y = ax^3 + bx^2 + cx + d$ best describes the relation between the log of the actual molecular weights of cellulose tricarbanilate (y) and their retention volumes (x). The following coefficients were calculated: $a = -0.00057626$, $b = 0.047981$, $c = -1.5575$, $d = 22.6164$. These gave a fit with a multiple regression coefficient of $r^2 = 0.999$ and an error on estimation below 4%.

The analysis of cellulose tricarbanilates obtained from different treatments by size exclusion chromatography was performed with a liquid chromatograph equipped with an automatic injector (Waters, WISP 712) connected to an on-line computer (NEC APC IV) via a Waters Maxima 820 data system. Four stainless steel columns connected in series were used (300 x 7.5 mm) packed with PLgel spherical particules (Polymer Laboratories) of particule size 5-10 µm, and pore size of 50, 500, 10^4 and 10^5 Å respectively. Detection was made with a UV monitor at 254 nm. A constant flow rate through the system was maintained by a high precision pump (Waters 590). The following operating conditions were utilized: eluent, tetrahydrofuran (continuously bubbled with N_2 to prevent the formation of peroxides); flow rate, 1 mL/min; temperature, 40°C; injection volume, 100 µL; sample concentration, 3-6 mg/mL.

As an example, the Figure 3.3 shows the size exclusion chromatograms of commercial cellulose (Sigmacel) and a preparation of oligosaccharides obtained from cellulose hydrolysis.

Table 3.1 indicates the degree of polymerization calculated from the chromatograms using the universal calibration. The polydispersities are also indicated.

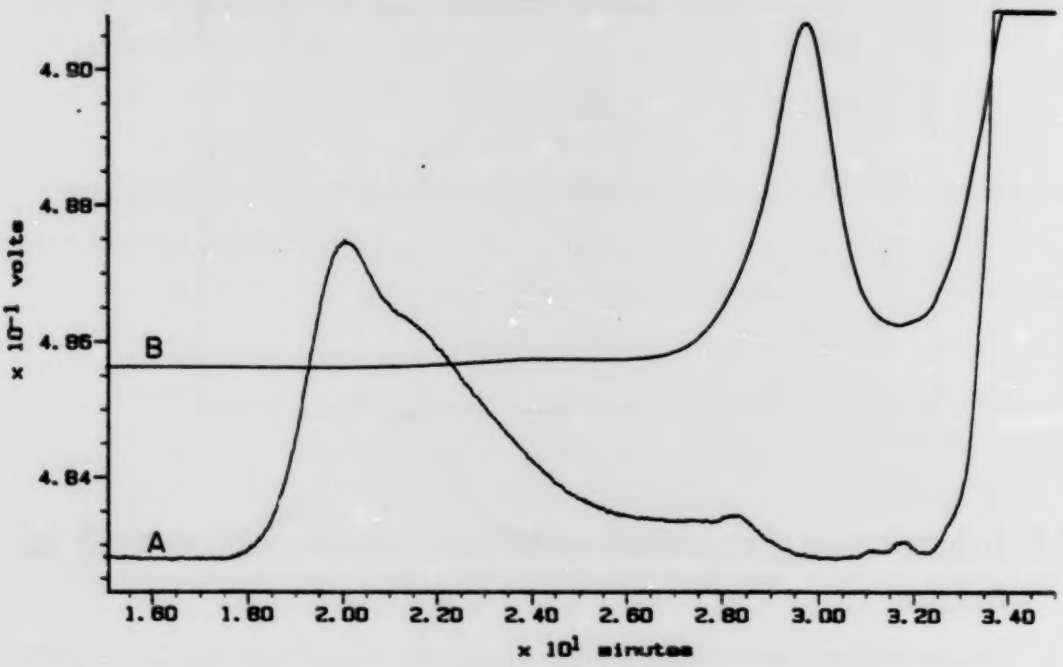

Figure 3.3: Size exclusion chromatography of tricarbanilates of (A) Sigmacel cellulose and (B) oligosaccharides

Table 3.1: Average degrees of polymerization and polydispersity coefficients of Sigmacel cellulose and oligosaccharides

	Sigmacel Cellulose	Oligosaccharides
$\overline{DP}_N$	156	7
$\overline{DP}_W$	1218	9
$\overline{DP}_Z$	2642	10
$\overline{DP}_W/\overline{DP}_N$	7.82	1.15
$\overline{DP}_Z/\overline{DP}_W$	2.17	1.18

3.2 Determination of crystallinity index and crystallite width by X-ray diffraction

Because it has been observed that cellulose is composed of amorphous and ordered regions, it is possible to determine the relative importance of the ordered region in a cellulose sample by means of X-ray diffraction. Effectively, an ordered region will diffract the light at precise angles due to specific planes of the ordered region. In the case of cellulose I, Segal *et al.*[17] have proposed in 1959 a method for the determination of a crystallinity index (CrI) based on the relative intensity of diffracted light measured at two angles:

- $I_{(002)}$ = diffraction intensity of plan (002) at 2θ angle = 22.6°
- I_{AM} = background at 2θ = 18.2°

The crystallinity index (CrI) is calculated by the following equation:

$$ICr = 100 \cdot \left[\frac{(I_{002} - I_{AM})}{(I_{002})} \right]$$

It is also possible to evaluate the width of the crystallites (Å) from the Scherrer equation[18] where K is a constant equal to 0.9, λ is the wavelength of the source and B is the width at the half-height in radian.

$$D = \frac{K\lambda}{B \cos\theta}$$

As an example, Figure 3.4 shows the diffractograms obtained from the allomorphs forms I and II of the cellulose:

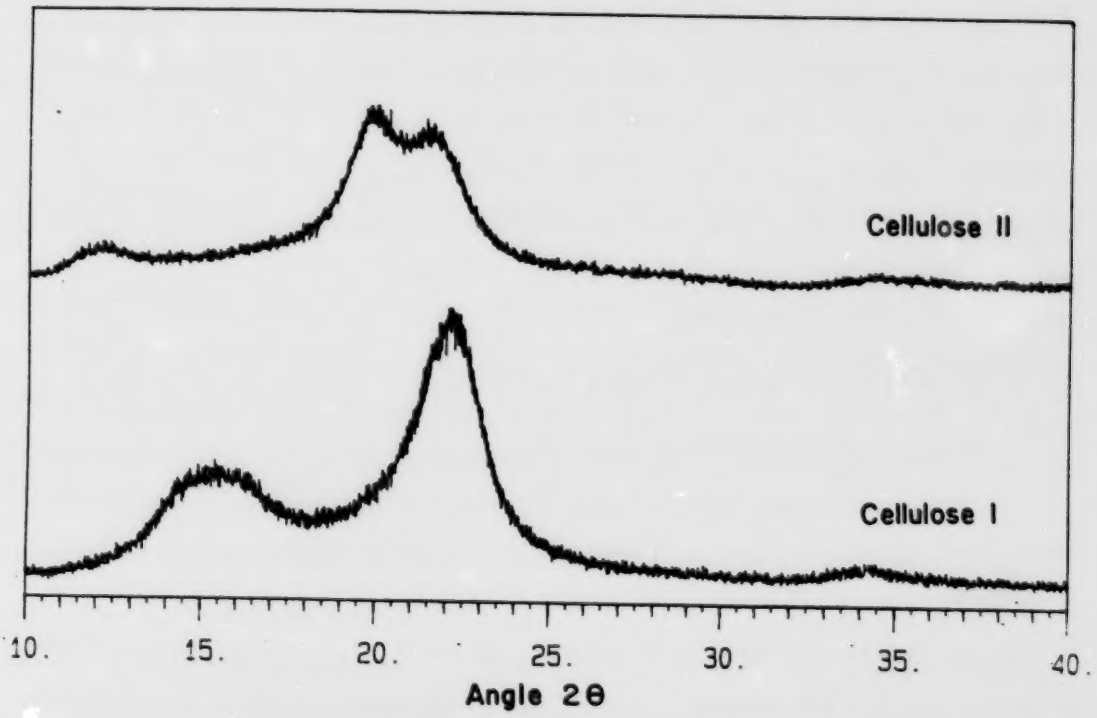

Figure 3.4: Diffractograms of celluloses I and II

The diffractometer used is a Rigaku "Geiger Slex" equipped with horizontal goniometer and a copper source (λ = 1.542 Å) to which a 40 mA current at a potential of 30 kV is applied. A nickel filter is used to eliminate the kβ. Diffraction spectra are recorded between 10 and 40° of 2θ angle at a speed of 3°/min. The cellulose sample (about 200 mg) is simply pressed in the window of the sample holder.

3.3 ^{13}C nuclear magnetic resonance spectroscopy (NMR)

3.3.1 ^{13}C NMR of lignins in liquid state

The spectra of the acetylated samples of glycol lignins and its fractions were obtained using a Bruker 250 WM Fourier Transform spectrometer. Samples were prepared by dissolving *ca.* 800 mg of lignin in 2 mL of deuterated dimethyl sulfoxide (DMSO-d_6) and placing the solution in 10 mm i.d. glass tubes. These were run at a temperature of 323°K. The instrument was set in proton inverse gated decoupled mode and the delay between scans was 2 seconds in order to allow complete relaxation of the carbons. The numbers of scans was 6000.

3.3.2 ^{13}C NMR of cellulose in solid state using cross polarization and magic angle spinning (CP/MAS)

The cross polarization technique, developped by Pines *et al.*[19-21] in 1972, allows us to obtain good spectra from a solid sample by means of the transfert of the magnetization of the proton to the carbon which increases the signal to noise ratio if the Harthmann-Hahn condition[22] is respected. However, the spectrum is very large and shows very poor resolution under these conditions because of the anisotropy of the chemical shifts. A very rapid rotation of the sample at the magic angle[23] will reduce the anisotropic pattern to a narrow signal at the mean of the chemical shift. This assures a strong enhancement of the resolution. The advantages of the PC/MAS technique are the following[24]:

- the cross polarization technique increases the signal to noise ratio and bypasses the relaxation process of the dilute nucleus;
- the high power decoupling of the protons during acquisition eliminates the dipolar interactions;
- the magic angle spinning eliminates the anisotropy of the chemical shifts in solids.

A scientific collaboration with Dr Van Calsteren of the Food Research and Development Center in St-Hyacynthe made possible the analysis of our samples by CP/MAS NMR and the procedure used was the following:

The spectra were recorded using a Bruker AM-300 instrument equipped with an MSL high-power amplifier, a MAS-DB pneumatic unit and a double-bearing probe. The respective frequencies for ^{1}H and ^{13}C were 300.13 and 75.47 MHz. About 500 mg of cellulose sample were pressed in an alumina rotor of 0.345 cm^3 to which a 5 kHz spinning rate was applied at a temperature of 308°K. The pulse sequence was a single contact and the contact time was fixed to 2 ms after optimization for our samples. The length of the $\pi/2$ pulse was 6 μs. The recycle time was of 8 s and detection was made with quadrature. Phase alternation was used throughout the signal acquisition to minimize baseline and intensity artefacts. The magic angle was adjusted by monitoring the ^{79}Br spectrum of KBr. A 15 Hz line broadening and one level of zero-filling were introduced prior to Fourier transformation to increase the signal to noise ratio. The chemical shifts relative to tetramethylsilane were determine by using the CH peak of adamantane at 38.3 ppm as an external standard. Between 1000 and 5000 scans were co-added.

As an example, the spectra of cellulose I and II are presented in Figure 3.5 and the assignments of the different peaks are presented in Table 3.2 based on the proposal of Isogai *et al.*[25].

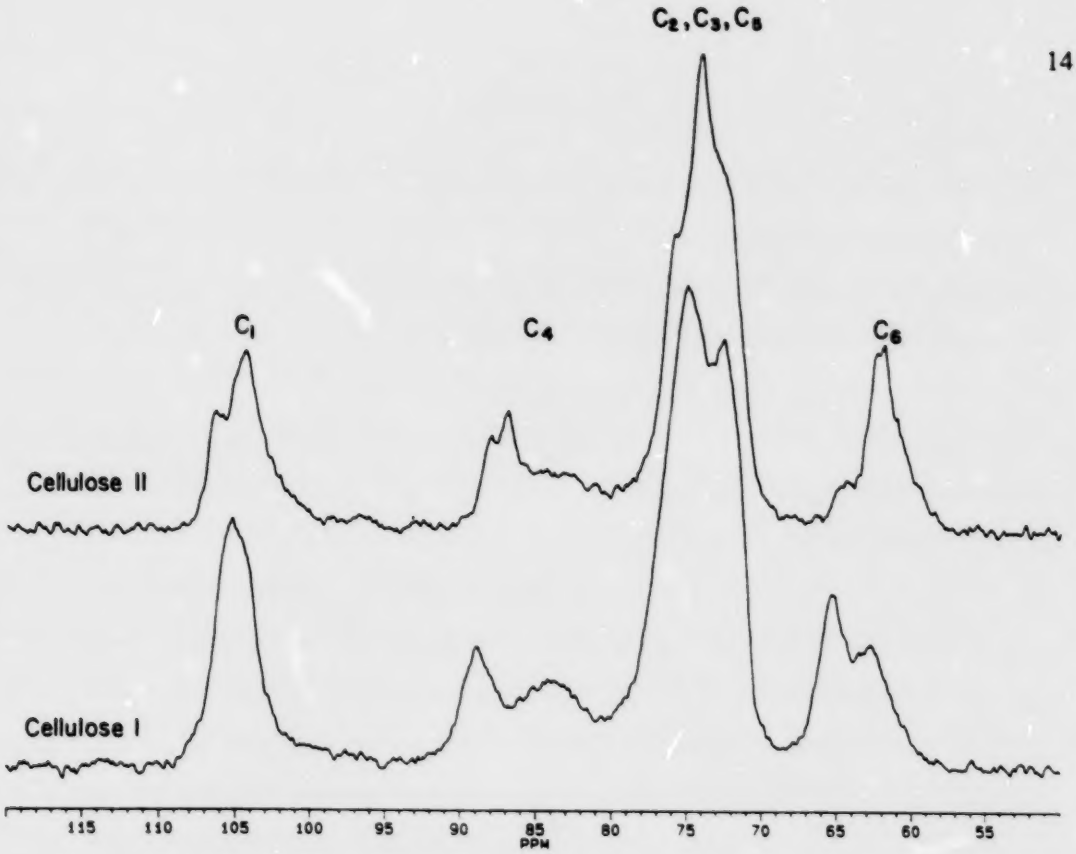

Figure 3.5: ^{13}C CP/MAS NMR spectra of cellulose I and II

Tableau 3.2: Chemical shifts and assignments of cellulose I carbons

Chemical shifts (ppm)	Assignments
105.3-106.0	C-1
89.1-89.8	C-4
84	C-4 (amorphous)
72-76	C-2, C-3, C-5
65.5-66.2	C-6
63	C-6 (amorphous)

4: FRACTIONATION AND HYDROLYSIS OF SOFTWOOD

In recent years, increased attention has been given to the so-called physical pretreatment techniques such as grinding, milling, irradiation and steaming of lignocellulosics without the addition of chemicals in order to meet different objectives which can be grouped as follows:
-the production of low cost sugars for use in biotechnological routes to fuels and chemicals;
-an increase of the accessibility of the cellulosic carbohydrate to cellulases with the goal of producing glucose for fermentation purposes;
- the use of low cost residual lignocellulosics as feed for ruminants.

A more recent objective of steam and aqueous pretreatments has been the fractionation of lignocellulosics to obtain each of the polymeric components in maximum yield and purity.

The visible physical effect of the steaming processes is the separation of lignocellulosics into individual fibres and bundles. However, by examining the changes in the ultrastructure and chemical composition of each constitutive component, it is clear that the steaming processes are chemical in nature, with hydrolysis as the predominant mechanism[26]. Steam/aqueous treatments can be considered as thermomechanochemical processes since heat (via steam addition), shear forces (induced by internal devices or through sudden decompression), and hydrolysis (with or without added acids or bases) are combined to destructure the lignocellulosic matrices. Two categories of processes are distinguishable:

1. treatments in which a large vapour phase is in contact with a wet solid. The latter is expelled out of the reaction vessel through intermittent decompression. These are the so-called steam explosion processes or thermomechanical-vapour treatments (TMV). Detailed descriptions of these processes are well known.[27-30]

2. treatments in which biomass is suspended as a slurry in water. Steam is usually employed to directly heat the mixture. Continuous systems as well as percolation reactors have also been well described.[1, 31-33]

The fundamental principles leading to autohydrolysis of the hemicellulose in hardwoods are well established[34,35] and kinetic models describing the solubilization profiles of the hemicellulose have been proposed and experimentally proven.[36,37] Less well understood are the structural and functional modifications of the lignin and cellulose as a function of the severity of the steam/aqueous treatment. Recently, a large effort has been devoted to chemical characterization of steam-exploded wood[38-40]. When comparing the results obtained by these authors using a severity factor which combines temperature and time of reaction as a unique parameter, we can conclude that the different steaming processes give similar results[26]. As an example, the severity maps well the different proportions of hemicellulosic polymers, oligomers, monomers and degradation products (furfural) obtained in the soluble phase.

Our study has been done using a different approach than steaming the wood. The aqueous fractionation process, previously described by Koeberle *et al.*[1], is based on a methodology in which an aqueous suspension of finely divided lignocellulosics at medium solids consistency is rapidly heated by steam addition and passed through homogenizing valves.[3] Ultrastructural modifications (defibration and defibrillation) can be induced by the mechanical treatment and the sequential fractionation of the main constituents of lignocellulosics can be achieved[41] while destructuring the cellulose matrix which results in increased accessibility of the cellulose to cellulases.[42] This technology has the advantage of very fast heating up and quenching periods (<10 sec) insuring a perfect control of the reaction time. Contrary to steaming processes where the solubles (hemicellulose) are left in contact with the fibers all along the reaction and have to

be subsequently washed from the fibers, the aqueous fractionation process provides rapid solubilization during the reaction, a constant removal of the solubles from the fibers and mass balances close to 100%.

It was felt that a complete description of how the constitutive polymeric fractions change as a function of the treatment severity for a generic steam/aqueous process was a major goal to pursue in order to better understand the principles of fractionation as compared to the steam-explosion processes. A secondary goal was to characterize the fractions and to provide basic guidelines for the possible use of the polymers present in the fractions.

4.1 Results obtained previously from fractionation of hardwood

The main focus of the previous contract[7] concerned the characterization of soluble and residual fractions of hardwood treated by aqueous/steam fractionation process at different severities. By treating *Populus deltoides* (a hardwood prototype) at different severities, the main objective was to obtain a detailed view of the changes of the constitutive polymers in the treated materials by analysis of the solid residual fractions[43] and the water-soluble fractions[44]. Characterization of the different constitutive polymers was carried out in order to correlate specific properties such as the: degree of polymerization; solubilization patterns; deacetylation; demethoxylation; thermal decomposition profiles; molecular weight distribution and chemical functionalities to the severity of the treatment.

The characterization of soluble and residual fractions obtained by steam/aqueous fractionation of *Populus Deltoides* as a function of the severity led to the following conclusions:

Primary conclusions:

At equivalent severity of treatment the aqueous/steam fractionation process is comparable to the steam explosion process with respect to yield of solubilization of hemicellulose and lignin or enzyme accessibility enhancement to substrates. There is a major difference, however, principally with respect to the autohydrolysis concept. Unlike the steam explosion treatment which provides a high yield of monomers, over 90% of the hemicellulose is solubilized as poly- and oligosaccharides. Even at the highest severity, very small amount of monosaccharides and only trace amounts of furfural are detected. The role played by the acetic acid in the autohydrolysis process is doubtful since no acetyl group is lost from either the residue or soluble fraction below a severity index of log R_0 = 3.5. Even at the highest severity used in our work (log R_0 = 4.3), over 75% of the acetyl groups remain with the hemicellulose. The hemicelluloses remaining on the wood fibers are richer in acetyl groups than those which are solubilized.

Secondary conclusions:

- The solubilization of nearly one third of the wood is possible at the highest severity used. The gaseous and volatile losses correspond to less than 6% of the initial material. At this severity, over 90% of the hemicellulose are solubilized along with a small fraction (<15%) of the lignin. However the maximum recovery of pentosans in the soluble fraction is 65% of the potential;

- The standard (ASTM & TAPPI) α-cellulose test has been demonstrated to fail as the severity of the pretreatment increases. By means of hydrolysis and quantification of the cellulose from the glucose content as recommended by Theander and Westerlund[33], we have confirmed that part of the cellulose fraction is soluble in sodium hydroxide solution. The change in method also requires that we "reconstitute" the hemicellulosic fraction from the monomers analysis;

- The fractionation process induces modifications in the thermal behavior of the residual lignin as shown by the DSC analysis. The extent of these modifications increases as a function of the severity and the patterns observed are typical of condensation reactions;

- Demethoxylation of lignin begins at a severity of log R_0 = 3.6 but even at the highest severity used in our work (log R_0 = 4.3), over 87% of the methoxyl groups remain with the lignin. We have shown that the demethoxylation occurs principally on the lignin present in the fiber and have proposed that this follows a pyrolysis mechanism rather than hydrolysis of the soluble lignin;

- The analytical methods for methoxyl and acetyl groups quantification underestimate their values when applied to native wood. We have shown that a destructuring treatment must be done in order to render these groups accessible to the reagents and therefore making them completely measurable;

- The extraction of the filtrate derived from the process by ethyl acetate enables the removal of more than half of the lignin-related phenolics present in the aqueous phase. The efficiency of the extraction can be followed by the disappearance of a unique exotherm (420-440°C) in the DSC thermograms;

- DSC and FTIR analyses show that the hemicellulose and lignin derived from aqueous/steam fractionation process have chemical functionalities and structural characteristics which are functions of the severity of the treatment;

- An important fraction of the wood inorganic matter (>80% at log R_0 = 4.3) is solubilized by the aqueous/steam fractionation treatment;

4.2 Results on fractionation and hydrolysis of softwood

4.2.1 Preliminary experiments

It is well known that the fractionation of softwoods by steam/aqueous processes is more difficult to conduct than that of hardwoods. In fact, we have found that the reaction temperature should be greater than 230°C in order to obtain suitable conversions and reach the objective of solubilization of a major fraction of the hemicellulose.

The main objective of the preliminary experiments carried out in collaboration with J. Salvado and N. Abatzoglou was to optimize the experimental conditions for fractionation of softwoods using very low concentrations of sulfuric acid either injected into the reactor or impregnated in the wood (sawdust) before the treatment.

The experiments were done with mixed species of coniferous wood milled to an average particule size of 0.5 mm and subsequently treated by the UdeS-F aqueous/steam fractionation process. The wood was suspended in water at a concentration varying between 7.0 and 7.2% (w/w). The concentration of sulfuric acid added to the suspension for the impregnation study was varied between 1.5 and 4 g/100g of wood. These give a final concentration of acid in solution between 0.11 to 0.28%. Recirculation of the suspension in the first section of the reactor was made for 15 minutes under nitrogen atmosphere at room temperature in order to homogenize the suspension. This also allows penetration of the acid in the wood and removes the dissolved oxygen. In one experiment, the recirculation time was reduced to 2 minutes. For each experimental point, around 120 to 130 g of wood goes through the main part of the reactor where sulfuric acid can be injected if it has not previously been added to the suspension. The same concentration range was used both for the injection and for the impregnation. The reaction time

was slightly above two minutes (125-140 sec.) and the suspension was automatically neutralized by addition of the necessary volume of sodium hydroxyde solution to a pH value around 4. The suspension was finally quenched rapidly to room temperature and atmospheric pressure.

Parameters studied with this experimental grid

- experiments 1-4: Treatment at 200°C with and without impregnation at two acid concentrations (0.15 and 0.2%)
- experiments 5-8: Treatment at 210°C with and without impregnation at two acid concentrations (0.13 and 0.24%)
- experiments 9-10: Treatment at 220°C with and without impregnation at an acid concentration of 0.27%
- experiments 11-12: respectively same parameters as in experiments 3 and 5 but the stainless steel tubular reactor had been replaced by one made with zirconium
- experiment 13: same parameters as in exp. 6 but the recirculation time has been reduced to 2 min. instead of 15 min.

For these preliminary experiments, the analytical methodology was quite simple and was constituted of:

- determination of the conversion, solubilized products and condensed products (vapor phase);
- determination of ashes and pH;
- HPLC analysis of the oligomers, monosaccharides and degradation products (furfural and HMF) in the liquid phase;
- HPLC analysis of the furfural present in the condensed vapor phase.

Results and preliminary conclusions

The first result, observed in all the experiments, is that the addition of very low concentration of acid allows us to decrease the temperature of the fractionation treatment by 30°C, from 230°C to 200°C.

The following conclusions, from the variation of each parameters studied with this experimental grid, can be drawn:

Increasing the acid concentration

-increases the glucose content (cellulose hydrolysis);
-increases the furfural content (xylose degradation);
-increases the HMF content (glucose degradation);
-decreases the oligomers content.

Increasing the temperature of the reaction

-increases the furfural and HMF yields (carbohydrate degradation);
-increases the conversion and the losses.

Effect of impregnation

We did not observe any conclusive effect of pre-impregnation of the wood with the acid. The total conversions and the yield of carbohydrates and degradation products are quite similar

with the wood previously impregnated and for the experiments where the acid was directly injected in the reactor. Our hypothesis is that the external impregnation procedure used is not efficient and that the acid is not able to reach the internal parts of the wood.

Effect of recirculation time

No effect has been detected and this is in agreement with the previous hypothesis, in which we suggested that the inner structure of the wood matrix is not impregnated by acid.

Effect of zirconium

The only observable result obtained from the use of zirconium instead of stainless steel in the tubular reactor is the drastic decrease of iron ionic species found in the soluble fractions. No evident effect on the fractionation process itself has been detected.

General conclusion

As a general conclusion for these preliminary experiments, it appears that the sulfuric acid impregnation does not seem to be a better way to perform the softwood hydrolysis than direct acid injection inside the reactor at elevated temperature. Based on this finding, the subsequent experimental work is divided in two parts:

1- Aqueous/steam fractionation of softwood **without acid addition**. These experiments were executed exactly as it had been described for fractionation of hardwood[7];

2- A temperature-dependent series of experiments of aqueous/steam hydrolysis of softwood using **addition of sulfuric acid** at very low concentration at the initiation of the high tem-

perature treatment.

4.2.2 Experiments, nomenclature and severity scale

4.2.2.1 Experiments in the process development unit (PDU)

The wood used is mixed species of *Picea* collected in the area of lac Mégantic, Québec, milled in order to obtain an average particle diameter of 0.5 mm and swelled 16 hours in water at a 6% w/w consistency. Recirculation of the suspension in the first section of the reactor was for 1 hour under nitrogen atmosphere at room temperature in order to homogenize the suspension and remove the dissolved oxygen. For each experimental point, 1.5 liters (containing ca. 150 g of wood) are pumped through the reactor system for a period of two minutes and quenched rapidly to room temperature. The reaction temperatures were varied from 180°C to 240°C. An additional experimental point at 60°C was obtained by water-extracting wood in a Sohxlet apparatus for 7 hours.

For the series of experiments using acid, the same conditions were used except that sulfuric acid is added just before the tubular reactor in order to reach a nominal acid concentration in the suspension of about 0.06 N. Sodium hydroxide is added at the end of the tubular reactor in order to neutralize the acid and obtain a solution with a pH around 5.

After filtration of the slurry obtained from the fractionation experiment, the residue was air-dried for 48 hours followed by drying periods at 110°C until constant weight was reached. The process filtrate was immediately lyophilized except for 50 ml aliquot which was kept frozen for pH determination and volatile acids analysis. The residue was water-extracted in a Sohxlet and the weight of the extractives was added to that of the filtrate for conversion calculation.

4.2.2.2 Correction of the acid concentration

In order to determine the amount of acid that is neutralized by the wood, we have made suspensions of 50 g of wood in sulfuric acid solutions at different acid concentrations and recorded the resulting pH after 0, 24 and 48 hours. The results obtained are presented in Table 4.1.

Table 4.1: pH of wood suspensions as a function of sulfuric acid concentration

Acid solution		pH of Wood Suspension		
Conc. (N)	pH	0 hours	24 hours	48 hours
0.004	2.47	4.12	4.16	4.20
0.01	2.32	3.10	3.10	3.13
0.02	2.12	2.60	2.40	2.40
0.04	1.90	1.90	1.96	2.20

From this Table, we were able to calculated that 100 g of wood will consume 8.4 ± 1.0 mmole of hydrogen ion. In the concentration range used for the experiments, 5 mmole/L of sulfuric acid is consumed due to the neutralization properties of the softwood. Consequently, we have substracted this amount from the acid concentration used for the hydrolysis experiments.

4.2.2.3 Nomenclature and severity scale

Time (t) and temperature (T) of each experiment can be combined into a single factor called Reaction Ordinate (R_0) as described previously[26]. The logarithm function of the reaction ordinate ($\log_{10} R_0$) is used as a severity scale for the thermomechanical treatment. This severity scale is calculated with the following equation:

$$R_0 = \int_{t_0}^{t_f} \exp\ [(T-100)/14.75]\ dt$$

In the present report, and in order to distinguish between no-acid (simple fractionation) and acid (hydrolysis) treatments, the different fractions are identified by the following code:

- F = the total softwood sample which has been fractionated;
- FR = the residual solid recovered after the fractionation treatment;
- FS = the soluble fraction found in the process filtrate after fractionation;

- H = the total softwood sample which has been hydrolysed;
- HR = the residual solid recovered after the hydrolysis treatment;
- HS = the soluble fraction found in the process filtrate after the hydrolysis.

The set of figures in the code refer to the treatment severity at which the particular sample was exposed. Thus the sample FS-3.8 represents the water-soluble fraction obtained from the wood fractionated without acid at a severity index of 3.8 (2 min at 220°C in our case).

4.2.3 Calculation of yields for both series of experiments

In order to calculate the mass balance of each experiment, we have to measure the weight of the following fractions:

- W_{S01}: weight of wood introduced in the reactor;
- W_{S02}: weight of wood left in the recirculation section of the reactor (untreated wood);
- W_{SP}: weight of material collected by washing the reactor after the experiment (treated

wood);

- W_{S11}: weight ofsolid residue obtained from the filtration of the slurry;
- W_{SD}: weight of dissolved solids obtained by freeze-drying of the soluble phase.

Using these numbers, the following parameters can be calculated:

$$\textit{Initial weight of wood fed to the process} = W_{S01} - W_{S02}$$

$$\textit{Yield of pulp} = \frac{W_{S11} + W_{SP}}{W_{S01} - W_{S02}}$$

$$\textit{Yield of solubles} = \frac{W_{SD}}{W_{S01} - W_{S02}}$$

$$\textit{Losses + volatiles} = 100 - (\textit{Yield of Pulp} + \textit{Yield of Solubles})$$

These parameters are presented in Tables 4.2 and 4.3 for the aqueous/steam fractionation mode (F series) and for the aqueous/steam hydrolysis mode (H series). For the calculation of the yield of solubles of the H series samples, we have substracted the amount of Na_2SO_4 produced by the neutralization of sulfuric acid and present in the freeze-dried filtrates.

Table 4.2: Yields obtained from aqueous/steam fractionation of softwood (F series)

	Temperature (°C)				
	60	200	220	230	240
log Ro	1.4	3.2	3.8	4.1	4.4
Initial (g)	---	121.7	139.7	138.3	138.5
Yield of pulp (%)	94.0	86.8	68.1	66.7	67.4
Yield of solubles	6.0	12.1	16.4	18.4	20.4
Losses and Volatiles	0	1.1	15.5	14.9	12.2

Table 4.3: Yields obtained from aqueous/steam hydrolysis of softwood (H series)

	Temperature (°C)				
	60	180	200	220	240
log Ro	1.4	2.4	3.2	3.8	4.4
H_2SO_4 conc. (N)	0	0.045	0.045	0.060	0.060
Initial (g)	---	150.3	115.1	123.7	125.6
Yield of pulp (%)	94.0	74.2	67.5	52.1	41.7
Yield of solubles	6.0	18.5	24.2	45.9	59.8
Losses and Volatiles	0	6.3	8.3	2.0	---

From these Tables and Figure 4.1 which shows the yield of solubles for the above series on softwood and the series carried out previously on hardwood as a function of the severity, the following conclusions can be drawn:

- The yields of solubles obtained via aqueous/steam fractionation mode is lower with softwood than hardwood. The amount of water-extractives is high (6%) and a fraction of them is probably lost as volatiles during the high temperature treatment. The yield of solubles reaches 20.4 g/100g of wood at the highest severity used;
- the yields of solubles are much higher in the presence of acid, ranging from 18% to 60% of initial wood but a conversion as high as 60% implies extensive hydrolysis of the cellulose;
- the amount of losses and volatiles which can be as high as 15.5% without using acid, is kept very low for the hydrolysis process. Acid catalysed condensation reactions of volatile products as well as conversion of volatile organic acids to salts can be part of the explanation to account for the low amounts of volatiles and losses observed during aqueous/steam hydrolysis of softwood.

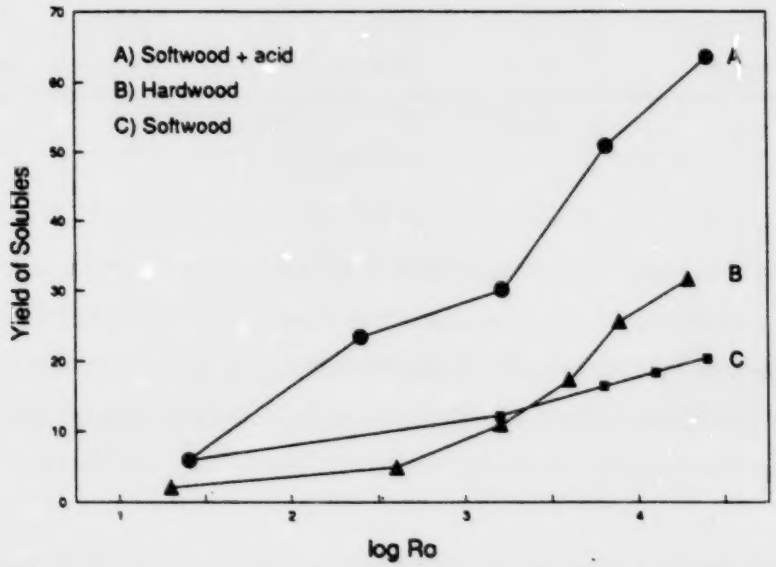

Figure 4.1: Yields of solubles obtained by aqueous/steam fractionation of hardwood and softwood and aqueous/steam hydrolysis of softwood as a function of the severity

4.2.4 Quantification of polymeric families present in softwood residues

Table 4.4 shows the data for the F series (without acid) using the standard ASTM procedure for determination of lignin, holocellulose and α-cellulose. Hemicelluloses have been quantified by difference (Holocellulose - (α-cellulose)).

Table 4.4: Yields of solubles and residual polymers (ASTM procedure) obtained by aqueous/steam fractionation of softwood (in g/100g of initial wood)

Fraction	F-1.4	F-3.2	F-3.8	F-4.1	F-4.4
Solubles	6.0	12.1	16.4	18.4	20.4
Hemicellulose	27.3	15.0	12.2	**22.1**	**16.5**
Cellulose	41.8	42.8	41.3	**28.2**	**32.1**
Lignin	25.6	29.2	30.9	30.7	30.8
Total	100.7	99.1	100.8	99.4	99.8

The only important conclusion from Table 4.4 is that an identical problem arises for the quantification of softwood fractionation as it was found for hardwood fractionation: a significant part of the cellulose seems to be solubilized following its gravimetric determination from the residue using standard procedures and is erroneously counted as hemicellulose for wood treated at severities beyond 4.0.

This effect of the aqueous/steam fractionation process seems to be general and we think that very careful attention should be given to results obtained from any kind of pretreatment before making any conclusion on the yields of hemicellulose solubilization from these treatments.

Consequently, we have decided to use the same method than for the aqueous/steam fractionation of hardwood. This method consists in a reconstitution of the hemicellulose and cellulose concentration in the softwood residues from the monosaccharides distribution obtained from the hydrolysis of the residues and analysis of the carbohydrates. Based on the monosaccharides distribution of the hemicellulose alone which can be obtained by the analysis of the carbohydrate present in the soluble phase, we are able to do this reconstitution. The discrepancies between the carbohydrates and the polymers quantification is caused by different factors: recovery factor of the carbohydrates after the post-hydrolysis, amounts of acetyl groups and uronic acids presents in the hemicelluloses. The mean general correction factors which should be used to convert the carbohydrate summations to polymer equivalents are the following:

hemicellulosic carbohydrates to hemicelluloses

1.39 ± 0.05

cellulosic carbohydrates to cellulose

1.10 ± 0.03

holocellulose carbohydrates to holocellulose

1.19 ± 0.03

These carbohydrate analyses will be presented in the next section (4.2.5) but Table 4.5 presents the quantification of the softwood polymers treated by aqueous/steam fractionation process obtained from this reconstitution and the following conclusions can be drawn:

- The solubilization of 20% of the wood is reached at the highest severity used in this process mode;
- an almost perfect correlation exists between the amount of wood and hemicellulose solubilized by the treatment; 20.2 g of hemicelluloses have been solubilized at the highest severity. This corresponds to 74% of the initial hemicelluloses;

Table 4.5: Yields of solubles and residual polymers (carbohydrate procedure) obtained by aqueous/steam fractionation of softwood (in g/100g of initial wood)

Fraction	F-1.4	F-3.2	F-3.8	F-4.1	F-4.4
Solubles	6.0	12.1	16.4	18.4	20.4
Hemicellulose	27.3	20.7	13.3	**8.2**	**7.1**
Cellulose	41.8	40.8	41.0	**42.1**	**41.5**
Lignin	25.6	29.2	30.9	30.7	30.8
Total	100.7	101.8	101.6	99.4	99.8

- the previous conclusion is validated by the fact that neither cellulose nor lignin have been solubilized. In fact, the amount of lignin present in the residues has increased by about 20% indicating the formation of a condensed product called "pseudo-lignin";
- the objective of fractionation has been reached by the fact that hemicelluloses seem to be the only polymeric component to be solubilized. However, we have to realize that the remaining fraction of hemicelluloses (26%) is harder to solubilize than the hemicelluloses from hardwood and accept that a partial solubilization is the penalty of the treatment without acids.

For the softwood treated by the aqueous/steam hydrolysis process, the standard ASTM procedure for cellulose has completely failed. Even the drastic nitric acid method (Kurschner) has given "foolish" results. The reconstitution from the carbohydrate composition is the only one which has given results with sense. Table 4.6 shows these results and leads to the following conclusions:

- the presence of acid (in the hydrolysis process mode) allows a complete solubilization of the hemicelluloses beyond a severity index of 3.2 but the cellulose begins to be solubilized at this

Table 4.6: Yields of solubles and residual polymers obtained by aqueous/steam acid hydrolysis of softwood (in g/100g of initial wood)

Fraction	F-1.4	H-2.4	H-3.2	H-3.8	H-4.4
Solubles	6.0	23.5	29.2	50.9	64.8
Hemicellulose	27.3	7.7	3.2	0	0
Cellulose	41.8	38.0	37.7	11.5	8.1
Lignin	25.6	31.2	30.2	36.0	25.9
Total	100.7	100.4	100.3	98.4	98.8

severity;

- as in the fractionation process mode, pseudo-lignin is clearly formed in the residue (see yields at severities 2.4, 3.2, 3.8) but extensive cleavage of the lignin carbohydrate complex must take place at the highest severity used (4.4) resulting in a "lignin yield" close to the initial lignin present in the wood;
- the total solubles as well as the composition of the residue from sample H-2.4 is close to the one obtained without acid at a severity of 4.4;
- the fractionation objective is reached at a severity of 3.2 where 89% of the hemicelluloses have been solubilized. This yield is superior to the best one obtainable without using acid. Beyond this severity, cellulose is hydrolysed.

4.2.5 Determination of carbohydrates composition

The monosaccharides distribution have been determined for all fractions obtained from F series and H series of experiments on softwood. These have been done by GLC of the silylated

hydrolysates after optimization of the hydrolysis conditions for each fraction. The results appear in Table 4.7 where the last column represents the sum of carbohydrates found in residues and filtrates for all experiments. The following conclusions can be drawn:

- the total concentration of carbohydrates (R + S) decreases from 56.1% to 50.8% as a function of the severity in the fractionation experiments while important degradation occurs in the acid hydrolysis experiments when the severity goes beyond 3.2. The carbohydrates recovery decreases below 20% for the highest severity (4.4) hydrolysis experiment;

- two hemicellulosic fractions can be identified in the fractionated samples: a first one which is solubilized as the severity increases until 3.8 and a second one which remains in the pulp even at the highest severity used (4.4);

- in the hydrolysed samples, the hemicelluloses are almost totally solubilized at a severity of 3.2. Beyond this point, glucose concentration in residues decreases as the cellulose is hydrolysed.

A condensed form of Table 4.7 is presented in Table 4.8 and gives the progression of experimental results which allows a maximum of hemicelluloses solubilization without inducing cellulose hydrolysis. As concluded in the previous section, the aqueous/steam hydrolysis of softwood at a severity of 3.2 seems to be the ideal condition in order to reach our fractionation objectives.

TABLE 4.7: Carbohydrate distribution among the soluble (S) and residual (R) fractions (units are in g/100g of initial wood) of the fractionated (F) and acid hydrolysed (H) softwood

Sample	Fraction of Wood	Arabinose	Xylose	Mannose	Galactose	Glucose	Total R or S	Total R+S
F-1.4	94.0	0.8	4.4	8.1	4.1	38.7	56.1	56.1
FR-3.2	87.9	0.4	3.7	8.0	2.4	37.0	51.4	57.5
FR-3.8	83.6	---	2.1	3.5	2.6	38.5	46.7	54.7
FR-4.1	81.6	---	0.9	2.1	1.5	36.1	40.6	49.0
FR-4.4	79.6	---	0.8	1.8	1.3	38.0	41.8	50.8
FS-3.2	12.1	0.8	1.7	2.4	0.3	0.9	6.1	
FS-3.8	16.4	0.4	2.6	3.3	0.4	1.3	8.0	
FS-4.1	18.4	0.2	2.7	3.5	0.4	1.5	8.4	
FS-4.4	20.4	0.1	2.5	4.1	0.5	2.0	9.0	
H-1.4	94.0	0.8	4.4	8.1	4.1	38.7	56.1	56.1
HR-2.4	76.5	0.1	1.6	1.9	1.2	38.7	43.5	53.2
HR-3.2	70.8	---	0.5	1.2	0.7	38.3	40.7	49.8
HR-3.8	49.1	---	---	---	0.1	11.7	11.8	20.4
HR-4.4	35.2	---	---	<0.1	---	8.2	8.2	16.7
HS-2.4	23.5	0.6	2.2	3.8	1.4	1.7	9.7	
HS-3.2	29.2	0.2	1.2	2.4	1.0	4.3	9.1	
HS-3.8	50.9	0.2	1.1	2.0	1.0	4.2	8.6	
HS-4.4	64.8	0.1	0.3	1.2	0.5	6.5	8.5	

Table 4.8: Carbohydrate yield for selected samples from fractionation and hydrolysis experiments on softwood (units are in g/100g of initial wood)

Sample	Conversion	Carbohydrates		
		Soluble	Residual	Total
F-1.4	6.0	---	56.1	56.1
F-3.2	12.1	6.1	51.7	57.5
F-3.8	16.4	8.0	46.7	54.7
F-4.1	18.4	8.4	40.6	49.0
H-4.4	20.4	9.0	41.8	50.8
H-2.4	23.5	9.7	43.5	53.2
H-3.2	29.2	9.1	40.7	49.8

4.2.6 O-acetyl groups analyses

The quantification of acetyl groups has been done by acidolysis of acetyl groups using oxalic acid and quantification of the acetic acid formed by GLC as described by Solar *et al.*[46] Acetylsalicylic acid has been used for the calibration curve and a straight line with a correlation coefficient of 0.99936 has been obtained giving a standard error of 3.5%. The results are presented in Table 11 for fractionated and acid hydrolysed softwood where they are expressed in g/100g of initial wood. Table 4.9 leads to following conclusions:

- these analyses indicate that one on three of the hemicellulosic xylose is acetylated and that the concentration of acetyl groups in the softwood hemicellulose is 6.2%;
- the deacetylation of softwood begins rapidly and the maximum deacetylation (63%) is obtained at a severity of 4.1 by the fractionation process;
- the acid hydrolysis process mode increases the extent of deacetylation. At the highest severity (4.4), 16.5% of the acetyl groups still remain linked to xylose;

Table 4.9: O-acetyl groups distribution among the soluble (S) and residual (R) fractions (in g/100g of initial wood) of the fractionated (F) and acid hydrolysed (H) softwood

Samples	Residues	Solubles	Total	% of Deacetylation
Wood	1.70	---	1.70	0
F-3.2	0.62	0.30	0.92	45.9
F-3.8	0.27	0.48	0.75	55.9
F-4.1	0	0.62	0.62	63.5
F-4.4	0	0.61	0.61	64.2
H-2.4	0.70	0.78	1.48	13.0
H-3.2	0.01	0.69	0.70	58.8
H-3.8	0	0.68	0.68	60.0
H-4.4	0	0.28	0.28	83.5

A comparison between the deacetylation of hardwood treated by aqueous/steam fractionation process and our present data on softwood is presented in Figure 4.2 as the percentage of deacetylation as a function of the severity.

In the case of hardwoods, no deacetylation occurs before a severity of 3.8 and even at the highest severity (90% of solubilization of hemicelluloses) more than 75% of the acetyl groups remain linked to the hemicelluloses. Haw *et al.*[47] have also observed by ^{13}C NMR low deacetylation of red oak treated by RASH process. The deacetylation is more pronounced in the case of softwoods where 60% of deacetylation occurs at the highest severity. However, the acetyl content of softwoods is lower than hardwoods: 1.7% of initial wood as compared to 3.7% for hardwoods. A very small increase can be observed when acid is added during the treatment.

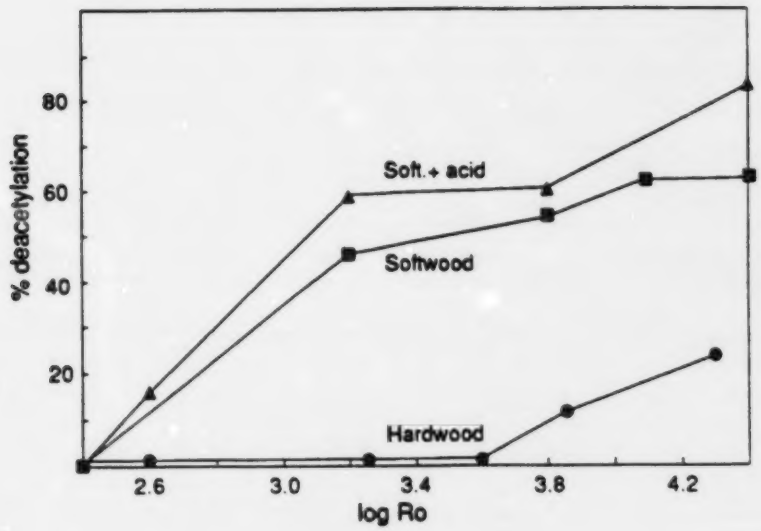

Figure 4.2: Deacetylation of wood hemicelluloses as a function of the treatment severity

Concerning the softwood, our results suggest that two mechanisms of deacetylation are present. The first one is a rapid deacetylation of the half of the acetyl groups which occurs at low severities. The subsequent deacetylation is much slower indicating the presence of a more resistant family of acetyl groups which are less accessible to hydrolytic attack.

4.2.7 Methoxyl groups analyses

The determination of the methoxyl groups among the soluble and residual fractions of softwood treated by aqueous fractionation and acid hydrolysis processes is presented in Table 4.10 and leads to the following conclusions:

- the fractionation process removes 5% of the methoxyl groups whatever the severity used. These methoxyl groups are probably linked to low molecular weight phenolics and not to

Table 4.10: Methoxyl groups distribution among the soluble (S) and residual (R) fractions (in g/100g of initial wood)

Samples	Residues	Solubles	Total	% of Demethoxylation
Wood	5.43	---	5.43	0
F-3.2	4.85	0.29	5.14	5.4
F-3.8	4.78	0.38	5.16	5.1
F-4.1	4.84	0.36	5.20	4.3
F-4.4	4.88	0.31	5.19	4.5
H-2.4	4.76	0.27	5.03	7.4
H-3.2	4.77	0.30	5.07	6.7
H-3.8	3.81	0.35	4.16	23.4
H-4.4	3.98	0.40	4.38	19.4

polymeric lignin;

- the same behavior is observed for the acid hydrolysis experiments until a severity of 3.2. Beyond this severity, demethoxylation increases to 20% and probably involves true demethoxylation of lignin macromolecules.

4.2.8 Determination of the crystallinity of the residues

The crystallinity index (CrI) has been determined following the method proposed by Segal *et al.*[48] from the X-ray diffraction patterns of the samples using the following equation:

$$CrI = 100\left[\frac{I_{002} - I_{am}}{I_{002}}\right]$$

where I_{002} and I_{am} are the intensities of the peak for the 002 plane ($2\theta = 22.5°$) and the amorphous fraction ($2\theta = 18.5°$), respectively. The CrI of the residues obtained by fractionation and hydrolysis is indicated as a function of the severity in Table 4.11 and leads to the following conclusions:

Table 4.11: Crystallinity index (%) of residues obtained by fraction and acid hydrolysis processes as a function of the severity

Log R_0	Crystalinity Index (CrI)	
	F Series	H Series
1.4	69.1	69.1
2.4	---	75.5
3.2	72.9	76.4
3.8	76.7	76.4
4.1	77.7	---
4.4	81.1	55.4

- the aqueous fractionation of softwood causes an increase of the crystallinity of the cellulose from 69 to 81% as it has been observed in the steam explosion process;
- the same tendency occurs for the acid hydrolysis process until a severity of 3.8. Beyond this severity, degradation of cellulose occurs and the crystallinity decreases. A description of the degradation mechanism can be found in section 5.1

4.2.9 Cellulase activity on treated residues

The cellulases saccharification yields (in g of glucose/100g of either residue or cellulose) have been determined for periods of 24 and 48 hours of incubation and the results are shown in Table 4.12 as a function of the severity.

Table 4.12: Cellulase activity expressed as glucose potential for softwood residues obtained by aqueous fractionation or acid hydrolysis processes

log Ro	Glucose potential (%)							
	Fractionated samples				Hydrolysed samples			
	residue basis		cellulose basis		residue basis		cellulose basis	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
1.4	6	7	10	14	---	---	---	---
2.4	---	---	---	---	6	9	11	17
3.2	15	16	31	32	5	10	9	18.1
3.8	15	19	31	40	9	14	34	41
4.1	16	20	33	41	---	---	---	---
4.4	17	21	36	45	11	12	38	44

Saccharification yield for native wood is below 15% of the glucose potential indicating that there is a structural barrier constraining the saccharification. The elimination of this structural barrier is maximized by the aqueous/steam fractionation process at a severity index of 4.4 where the yield of saccharification reaches 45% of the cellulose present for 48 hours of incubation. In the case of the acid hydrolysis process, the presence of acid seems to inhibit the effect of destructuration to enzyme accessibility until the severity reaches the value of 3.8. At

this severity, cellulose has begun to be hydrolysed during the process and, as a consequence, the accessibility increases in order to reach the glucose potential value obtain for the aqueous/steam fractionation process. However we should note that large part of the cellulose has already been solubilized by the treatment beyond a severity of 3.2. In Table 4.12, glucose potential is expressed in g/100g of residue (residue basis) and also in g/100g of cellulose present in residue (cellulose basis).

4.2.10 Solvent extraction of the solubles and phenolics distribution

The process filtrates (solubles) obtained from the aqueous/steam fractionation and acid hydrolysis of softwood are mainly composed of hemicellulose and phenolics derived from lignin. In order to separate these two "families", an extraction of the hydrophobic fraction was carried out by two sequential steps consisting of mixing of the lyophilized solids (1g) with pure ethyl acetate (50 ml) for two hours, each step.

Quantification of the total water-soluble lignin-derived phenolics was made by a modified version of the Folin-Denis spectrophotometric method for quantification of phenol. The modification is that the calibration curve is made with tannic acid which may be considered as a "prototype phenolic material"

The results of solvent extraction are presented in Table 4.13 where the organic fraction (S_{OP}) has been separated into volatile and non-volatile products (at 60°C under vacuum). The results are expressed on g/100g of dissolved solids recovered in the soluble fractions. The dissolved material present in the organic phase extracted (S_{OP}) represents always less than 7% of the process filtrate (S). The yield of extraction represents about half of that obtained by

treating a hardwood species (*Populus deltoides*) under the same conditions. Before giving any conclusion from this observation, we have to examine the distribution of the phenolics in the solubles (S), aqueous (S_{AP}) and organic(S_{OP}) fractions.

Table 4.13: Separation of the process filtrate (S) into an aqueous phase (S_{AP}) and an organic phase (S_{OP}). Yields are expressed in g/100g of dissolved solids in the process filtrate.

Solubles	Aqueous fraction	Ethyl acetate fraction (S_{OP})		
(S)	(S_{AP})	Non-volatile	Volatile	Total
FS-3.2	98.0	1.1	0.9	2.0
FS-3.8	98.1	1.1	0.8	1.9
FS-4.1	97.5	1.7	0.8	2.5
FS-4.4	93.3	2.1	4.6	6.7
HS-2.4	94.0	0.4	5.5	5.9
HS-3.2	97.3	0	2.7	2.7
HS-3.8	---	---	---	---
HS-4.4	96.9	0.3	2.8	3.1

The distribution of the phenolic products in the solubles (S), aqueous (S_{AP}) and organic (S_{OP}) phases is shown in Table 4.14 for both fractionation and hydrolysis series. The data must be interpreted as tannic acid equivalent instead of lignin-derived phenolics.

The concentration of phenolics in the soluble fractions obtained from fractionation of softwood represents 10 % of the total dissolved solids. Less than 12 % of these phenolics are extracted by ethyl acetate in all the samples while about 90% remain in the aqueous phase. The

non-extracted phenolics are too hydrophilic or possibly linked to carbohydrates as lignin-carbohydrates complexes (LCC). In the case of hardwood, the concentration of phenolics in the soluble fractions obtained from fractionation is equivalent (10%) but more than the half of these phenolics are extracted by ethyl acetate in all these samples. These results suggest that the inefficiency of the ethyl acetate extraction of the solubles obtained by the fractionation of softwood is not caused by a smaller concentration of hydrophobic components (phenolics) but by the fact that these phenolics are probably more strongly associated with carbohydrates than in the case of hardwoods.

Table 4.14: Phenolic content of the filtrates derived from the process (S) and of their corresponding aqueous and organic phases (S_{AP} and S_{OP}). In g/100g of dissolved solids in the process filtrate

Sample	Phenolics			Extraction
	S	S_{AP}	S_{OP}	Yield (%)
FS-3.2	10.5	9.5	1.0	9.5
FS-3.8	10.0	9.3	0.7	7.0
FS-4.1	9.8	9.1	0.7	7.1
FS-4.4	10.1	8.9	1.2	11.9
HS-2.4	2.1	1.3	0.8	38.1
HS-3.2	2.3	1.5	0.8	34.8
HS-3.8	3.4	2.6	0.8	23.5
HS-4.4	4.9	3.9	1.0	20.4

The concentration of phenolics in the soluble fractions obtained from acid hydrolysis of softwood increases from 2.1 to 4.9 % which is lower than for soluble fractions obtained by

aqueous/steam fractionation. While less than 10 % of these phenolics are extracted by ethyl acetate in the samples from fractionation, the extraction yield varies between 20 to 40% in the hydrolysed samples. The soluble phases obtained from acid hydrolysis of softwood are less rich in phenolic compounds but the same total fraction (1g/100g of dissolved solids) than in the fractionation mode can be extracted by ethyl acetate. As a general conclusion, contrary to soluble fractions obtained from hardwood, the ethyl acetate extraction step is unnecessary in the case of fractionation or acid hydrolysis of softwood.

4.2.11 Thermal analyses of treated residues

An indication of the influence of the treatment severity on thermal behavior of polymers is given in Figure 4.3 showing the thermograms of the initial wood and of the residues obtained by DSC under air atmosphere from 150 to 500°C. The important exotherm at 320-323°C is related to cellulose thermal degradation and seems unaffected by the steam/aqueous treatment. The small exothermic deviation of the baseline between 250 and 300°C, attributed to the presence of hemicellulose, decreases as a function of the severity.

The second exotherm in the 380-490°C area is mainly related to thermal degradation of the lignin. The maximum temperature of the lignin exotherm varies as a function of the severity of the treatment. The temperature of the maxima increases from 437°C to reach 480°C for the treatment at the highest severity. The variation of the lignin exotherm suggests a chemical and/or physical transformation, probably due to condensation reactions, of the polymer during the steam/aqueous treatment.

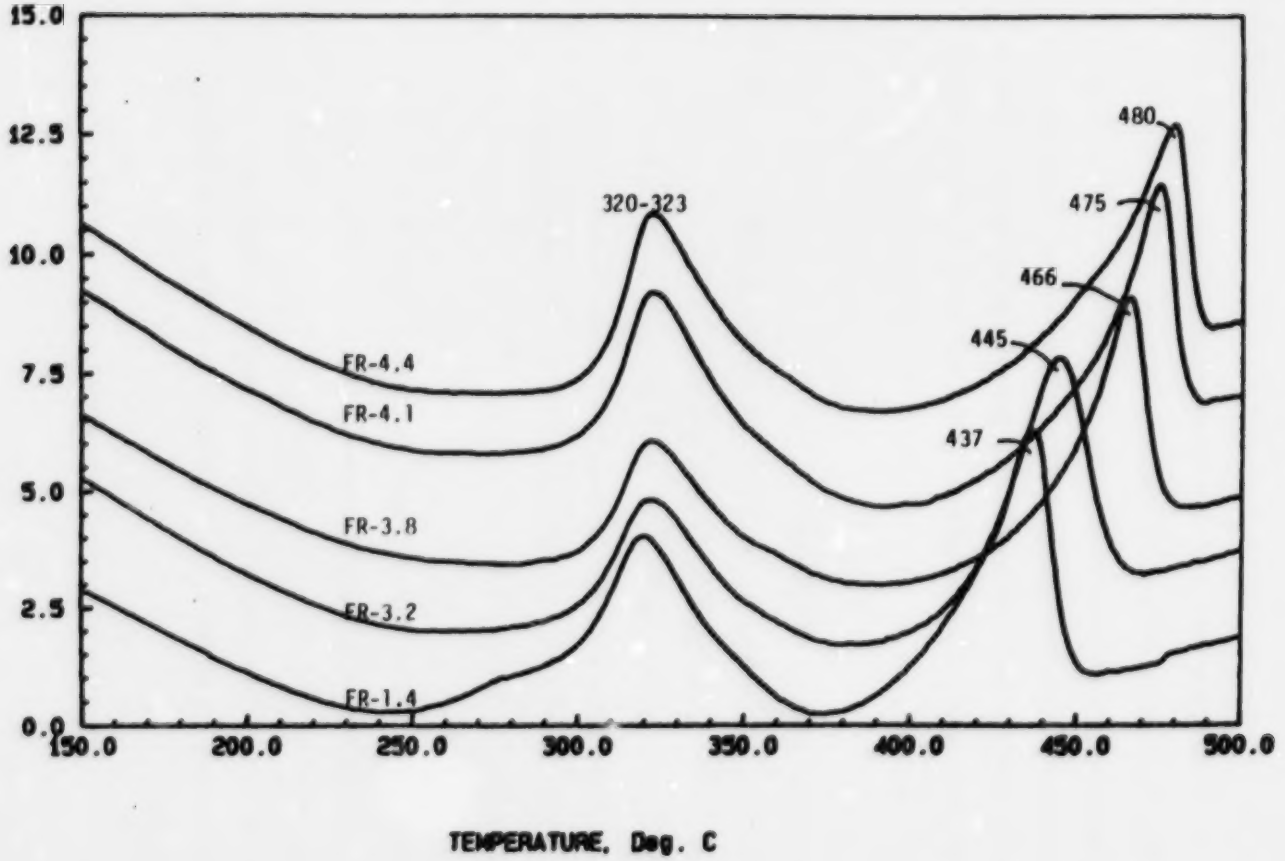

Figure 4.3: Differential scanning calorimetry (DSC) in air atmosphere of softwood residues obtained from aqueous/steam fractionation process

4.2.12 Infrared analyses of treated residues

The infrared spectra of initial wood and residues of both seris have been recorded by diffuse reflectance from 4600 to 400 cm^{-1} but the 4000-2000 cm^{-1} and 800-400 cm^{-1} areas do not contain substantial information regarding the hemicellulose and lignin fractionation. In fact, the large hydroxyl vibrations (3400 cm-1) and the CH vibrations (3000-2700 cm-1) are identical for all residues and have been omitted. Consequently, the spectra of the initial wood and the residues treated in fractionation mode shown in Figure 4.4 cover the 1850-750 cm-1 area where the relevant information is located. The major peaks related to the different hemicellulose and lignin vibrations can be listed as following:

- 1735 cm^{-1}	xylan carbonyl stretching
- 1655 cm^{-1}	keto-carbonyl conjugated with aromatic ring
- 1595, 1510 cm^{-1}	benzene ring stretching in lignin
- 1450 cm^{-1}	CH_3 deformation in lignin and CH_2 bending in xylan
- 1430 cm^{-1}	vibration of secondary alcohol
- 1370 cm^{-1}	CH_2 bending in hemicellulose
- 1335 cm^{-1}	lignin vibration
- 1320 cm^{-1}	lignin vibration
- 1270, 1235 cm^{-1}	guaiacyl and syringyl in lignin, C=O in hemicellulose
- 1160 cm^{-1}	C-O-C asymmetric band in hemicellulose
- 1112 cm^{-1}	O-H association band in hemicellulose
- 1060 cm^{-1}	C-O stretching of secondary alcohol
- 1035 cm^{-1}	C-O stretching in hemicellulose
- 897 cm^{-1}	C_1 group frequency in hemicellulose
- 815 cm^{-1}	aromatic vibration

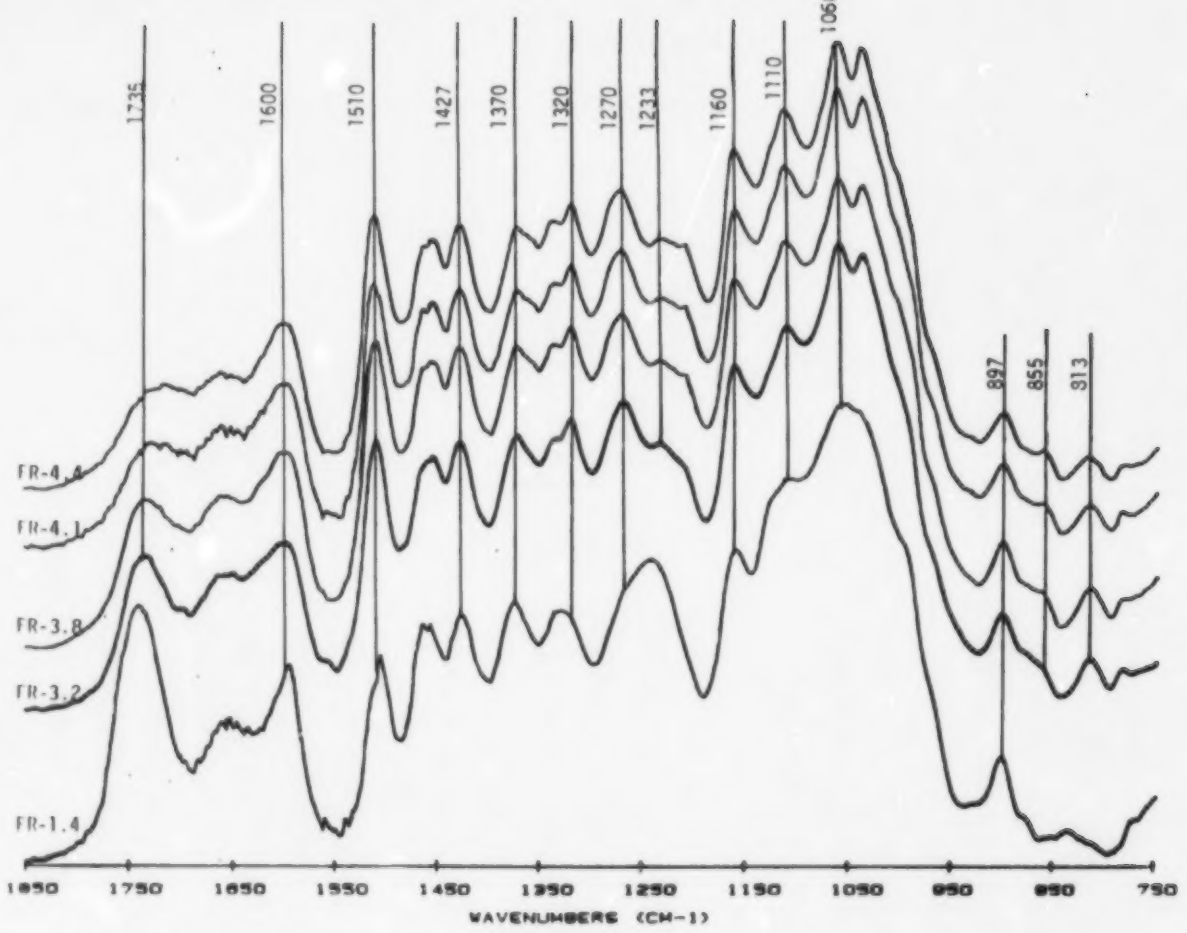

Figure 4.4: FTIR spectra of softwood residues obtained by aqueous/steam fractionation process (FR)

In order to facilitate the spectra comparisons in Figure 4.4, the peak of cellulose at 1060 cm^{-1} has been adjusted to the same height from the baseline as measured at 1900 cm^{-1} for all of the samples. Each spectra has been displaced vertically to clarify the comparisons. From these spectra, the solubilization of hemicellulose becomes evident and is supported by the decrease of the hemicellulose-related peaks at 1735, 1370, 1235 and 897 cm^{-1}. It is also possible to relate the enhancement of the cellulose peaks at 1112, 1060 and 1035 cm^{-1} to the disappearance of the hemicellulose vibrations at 1055 cm^{-1}.

Despite the fact that a portion of the lignin has been solubilized, all the lignin-related peaks appear to be unchanged or enhanced as the severity of the treatment increases. This can be explained by the fact that the lignin concentration in the residues increases as a function of the severity and we can conclude that the percentage of the lignin which is solubilized by the process is below the conversion percentage and that this tendency increases with the severity. This can be illustrated by the two large bands located at 1327 and 1240 cm^{-1}in the spectrum of initial wood which are resolved in two and three bands respectively in the treated residues.

The evolution of the infrared spectra of softwood residues obtained from the acid hydrolysis experiments can be summarized as follow:

- conservation of the lignocellulosic character;
- complete solubilization of the hemicellulose as observed by the disappearance of the hemicellulosic carbonyl band at 1745 cm^{-1};
- degradation of the cellulose begins at a severity of 3.8 and increases as demonstrated by the appearance of a carbonyl band located at 1710 cm^{-1} (see section 5.1 for complete assignment);
- increase of the aromatic character of the residue as indicated by the 1515 cm^{-1} band.

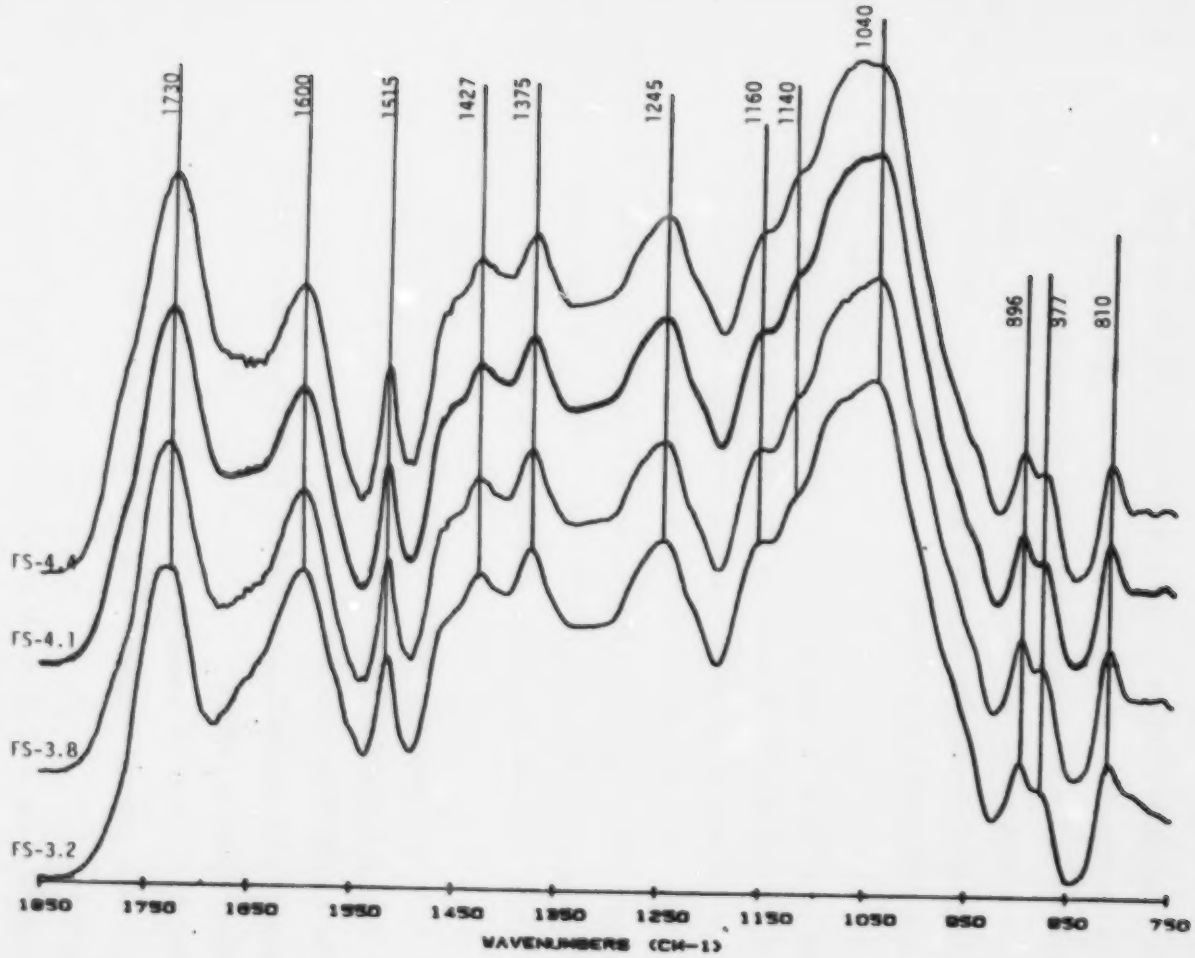

Figure 4.5: FTIR spectra of softwood solubles obtained by aqueous/steam fractionation process (FS)

Figure 4.5 shows the DRIFT spectra of the soluble fractions of the fractionated series as a function of the severity. All the spectra are similar indicating that even for the lowest treatment (FS-3.2) which has solubilized only 6% of the initial material, the composition of the solubles is a mixture of hemicellulose and phenolics.

In order to know the variation in the relative importance of the different bands, the band at 1040 cm^{-1} has been adjusted to the same height on all the spectra and it can be observed that all the bands remain relatively constant as a function of the severity except the 1725 and 1245 cm^{-1} bands which increase in intensity. Both are related to carbonyl vibration of the acidic groups present in hemicelluloses. This is an indication that the solubilized hemicelluloses have a stronger acidic character as the severity of the treatment increases. It is a well-known fact that for all fractionation process, the pH of the soluble phase decreases as a function of the severity. This has been attributed to the acetic acid liberated from the autohydrolysis of acetyl groups. We can now postulate that the pH variation could also be attributed to the acidic character of the hemicellulose itself.

FTIR spectra of the aqueous and organic phases obtained from the soluble phase after an extraction with ethyl acetate have also been recorded. It has been observed that the hemicellulose fraction solubilized by the treatment is quite homogenous all along the severity scale. The only detectable variation is the increase of the relative concentration of carbonyl groups as demonstrated by the increase of the height of the band at 1725 cm^{-1}. From the spectra of the ethyl acetate extracts (FS_{OP}), we have observed that the fingerprint of hemicellulose (1000-1200 cm^{-1}, 895 cm^{-1}) is only present in the lowest severity sample (S_{OP}-3.2). The other FS_{OP} fractions obtained from samples treated at higher severity are characteristic of lignin.

From the spectra of the soluble fraction of the hydrolysis series (HS, HS_{AP} and HS_{OP}) we can conclude that the soluble hemicelluloses are very degraded while the polyphenolics extracted by ethyl acetate seem to be undegraded and show the major lignin characteristics.

4.2.13 General conclusions on fractionation and hydrolysis of softwood

Primary conclusion

The efficiency of the aqueous/steam fractionation process is well recognized for the removal of hemicelluloses of hardwood. We have demonstrated that it is also possible to solubilize two thirds of the softwood hemicelluloses by this process. The addition of acid at very low concentration increases hemicelluloses solubilization to 90%. Treatments at higher severities induce the hydrolysis of cellulose.

Secondary conclusions

- The addition of very low concentration of acid allows us to decrease the temperature of the fractionation treatment by 30°C, from 230°C to 200°C for equivalent conversion. It appears that the sulfuric acid impregnation is not a better way to perform the softwood hydrolysis than direct acid injection inside the reactor at a point at which the suspension has reached the desired reaction temperature;

- As it was the case for hardwood, the standard (ASTM & TAPPI) α-cellulose test has been demonstrated to fail as the severity of the pretreatment increases. By means of hydrolysis and quantification of the cellulose from the glucose content as recommended by Theander

and Westerlund[33], we have confirmed that part of the cellulose fraction is soluble in sodium hydroxide solution. The change in method also requires that we "reconstitute" the hemicellulosic fraction from the monomers analysis;

- The deacetylation of softwood hemicelluloses is relatively more important than for hardwood but the low acetyl content of softwood does not lead to an important generation of acetic acid. In the range of severities which give high solubilization yields, the demethoxylation of the lignin is below 8%;

- A high index of crystallinity (81%) as well as an enhancement of the enzyme accessibility can be obtained from the cellulose derived from the aqueous/steam fractionated softwood.

5: DEPOLYMERIZATION OF CELLULOSE

5.1 Model for depolymerization by acid hydrolysis processes

5.1.1 Introduction

The hydrolysis of cellulose to glucose by dilute acid hydrolysis results in yields limited to 60-65% of the potential glucose. Many chemical models have been proposed to explain these yields[49,51], most of them being variations of Saeman's kinetic model which, in 1945, proposed the following sequence:

$$\text{Cellulose} \xrightarrow{k_1} \text{Glucose} \xrightarrow{k_2} \text{Degradation products}$$

None of the models considers the possibility of physico-chemical transformations of the cellulosic substrate itself with the extent of the reaction as being partially responsible for the observed limited glucose yields. The most recent model for dilute acid hydrolysis of cellulose[51] incorporates the reversion and degradation reactions of glucose and the presence of readily hydrolysable cellulose. In this model, the chemical transformation of glucose (i.e. degradation) appears to be the main reason for the low sugar yield observed in hydrolytic processes.

The starting hypothesis for our work involves a chemical transformation of a fraction of the cellulose itself in the polymeric state. This modified cellulose cannot give 100% glucose by further hydrolytic treatment. Figure 5.1 incorporates our hypothesis taking the model of Conner *et al.* as reference. Also we have added to the model the possible production of soluble oligomeric intermediates which have been found in non-negligible quantities during processes involving high temperature, low acid concentration and short residence time conditions[52].

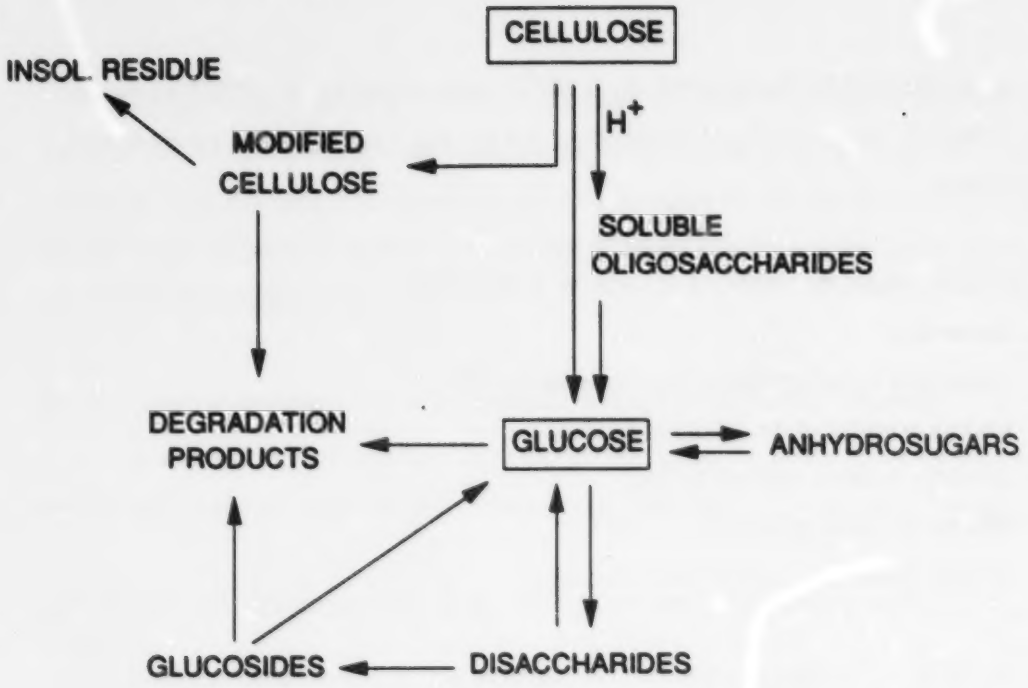

Figure 5.1: Proposed model for dilute acid hydrolysis of cellulose

A large fraction of our efforts has been completed and described previously[7,53]. The objectives of the present work were to completely characterize the cellulosic residues obtained at advanced stages of the acid hydrolysis. The characterization has been done for two series of experiments:

- Hydrolysis of cellulose using the standard analytical procedure with hydrochloric acid 2.5 N under reflux for reaction times varying between 0 and 48 hours; this process is considered as the low severity reference process;
- Hydrolysis in a process development unit previously described[52,54]. This process involves rapid passage of a cellulose slurry through a shear device and is followed by sulfuric

acid addition (0.08 N) and hydrolysis at 190°C in a plug flow reactor (PFR) for a reaction time varying between 0 and 70 minutes; this process is considered as a high severity process.

For each sample, the following analyses were conducted:

- conversion;
- viscosimetry average degree of polymerization ($\overline{DP}_v$);
- glucose potential of the cellulosic residue;
- thermogravimetric analysis (TGA);
- differential scanning calorimetry (DSC);
- infrared spectroscopy by diffuse reflectance.

The results on the depolymerization of cellulose by acid hydrolysis processes have led to the following conclusions:

The sugar potential of the unconverted cellulosic residues decreases with the extent of depolymerization. This behavior is more pronounced in the dilute acid process and significantly accounts for the reduction in overall sugar yield. Such an effect had led to the consideration that a new kinetic pathway for cellulose degradation needs to be introduced into the overall kinetic model for the prediction of conversion and sugar yields from dilute acid hydrolysis of cellulose.

Thermal analysis (TGA, DSC) and DRIFT can be used as tools to follow the changes of the cellulosic residues as a function of the extent of depolymerization. DRIFT data indicate that the pyranose ring structure of cellulose seems to be unaltered but the hydrogen and hydroxyl groups are affected by oxidative processes conducting to carbonyl formation.

The high severity dilute acid hydrolysis process modifies the cellulose structure in a different way than the lower severity process used as reference as determined by DSC but this difference is not reflected in the chemical structure as shown by infrared spectroscopy. This indicates that high severity processes induce modifications that are more related to structural than chemical rearrangements.

5.1.2 Complementary work executed during the present contract

Two series of experiments have been carried out during the present contract with commercial cellulose in the UdeS-H continuous process. The objectives were the following:

- confirm the chemical degradation of the residual cellulose in a continuous acid hydrolysis unit;
- study the effect of reaction temperature in terms of yield of solubles, degradation of the remaining cellulose and enzymatic accessibility;

The experimental conditions were the following: a suspension of cellulose (6%) in water is continuously recirculated in a first section of the process development unit. A fraction (10%) of the recirculating suspension is transferred to a second section of the unit where it is rapidly heated to the desired temperature (200-240°C) by steam injection. After passsage of the heated suspension through a homogeinizing valve, sulfuric acid is added in order to give an acid concentration of 0.5% or 1.0% by weight (series 1 and 2). The hydrolysis occurs then in a tubular reactor where the residence time has been fixed, for our experiments, to 2 minutes. Before flashing the suspension, a solution of sodium hydroxyde is injected in order to adjust the pH at about 5. The suspension is filtered and the solid cake is washed with water. Determination of products in the filtrate and the solid residual fractions is then done. The conversions obtained for both series as a function of the temperature of the reaction are shown in Table 5.1. These series of experiments gave a range of conversions from 24% to 95%.

TABLE 5.1: Conversions obtained from the hydrolysis of cellulose in the UdeS-H continuous process as a function of temperature (residence time = 2 min)

Temperature (°C)	Acid concentration	
	0.40-0.48%	1.1-1.3%
200	24.3	71.0
220	46.8	96.0
230	80.5	95.8
240	94.4	94.1

These series of experiments have been analysed following the same procedure than that used for previous experiments. Furthermore, we have added three new analytical methods to all these cellulosic residues. The following complementary results will be briefly discussed in the next section:

- determination of the molecular weight distribution by size exclusion chromatography. A description of the procedure is provided in Section 3.1.2;
- X-ray diffraction analysis following the procedure described in Section 3.2;
- solid state 13C NMR using cross polarization and magic angle spinning (CP/MAS). A description of the procedure is provided in Section 3.3.2.

5.1.3 Molecular weight distribution of acid hydrolysed cellulosic residues

Before discussion of the molecular weight distribution of cellulosic residues, a presentation of the glucose yields obtained from the different processes is of the essence. Figure 5.2

shows the process yields as a function of conversion. For the series H-An (analytical scale) and H-Di (discontinuous, batch, process), the yield is the summation of the glucose available in the residues and the amount of solubles. For the H-Co series (continuous process), the glucose in solubles has been quantified.

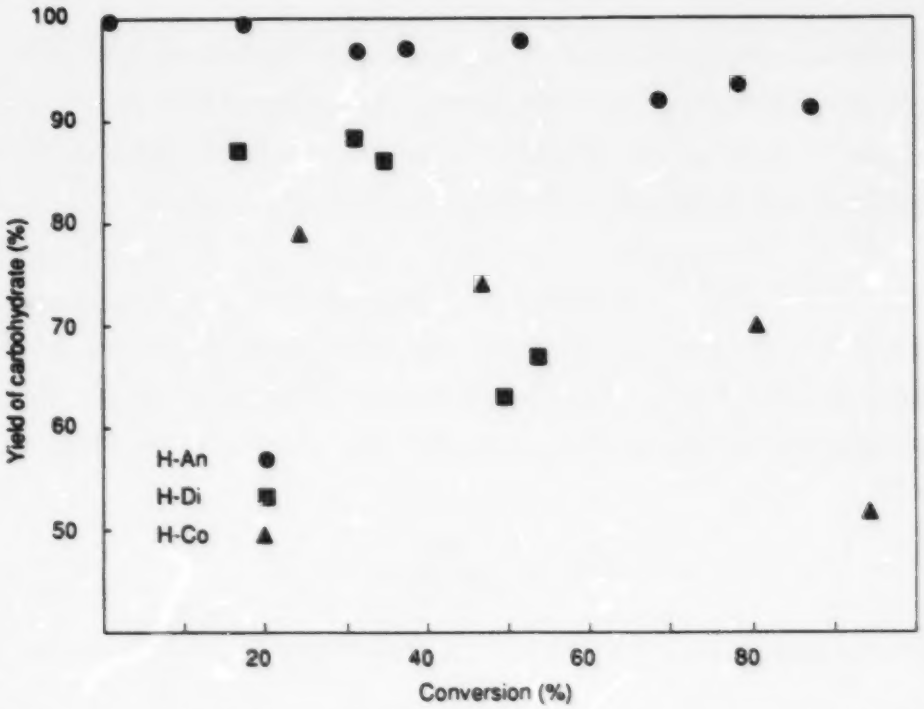

Figure 5.2: Carbohydrate yields for the three series of experiments as a function of conversion.

Figure 5.2 led to the following conclusions:

- the H-An series led to the highest yield with only 10% of the cellulosic residue which is degraded at a conversion of 87%;
- an increase in the temperature leads to degradation of the cellulose in the solid state;

- the degradation seems to be a two stage mechanism: a rapid degradation of 15-20% of the solid cellulose occurs at low conversions (<30%). Further rapid degradation occurs at conversions higher than 35% for H-An process and at conversions higher than 65% for the H-Co process;

The molecular weight distribution of cellulosic residues has been obtained by size exclusion chromatography of their carbanilate derivatives as described in Section 3.1.2. As an example, Figure 5.3 shows the chromatograms of initial cellulose, the residues of the H-An series (identified by their hydrolysis time) and of a mixture of oligosaccharides.

From these chromatograms, the average molecular weight by number ($\overline{DP}_n$), by weight ($\overline{DP}_w$) and by Z ($\overline{DP}_z$) have been calculated as well as the polydispersities and the ratio of these polydispersities (CPC). CPC is an index of the real molecular weight distribution as compared to a logarithmic distribution. A value of CPC greater than unity indicates an excess of molecules with DP > $\overline{DP}_n$.

$$CPC = \frac{\overline{DP^2}_w}{\overline{DP}_n \cdot \overline{DP}_z}$$

The results are presented in Table 5.2 for the residues of the three series of experiments.

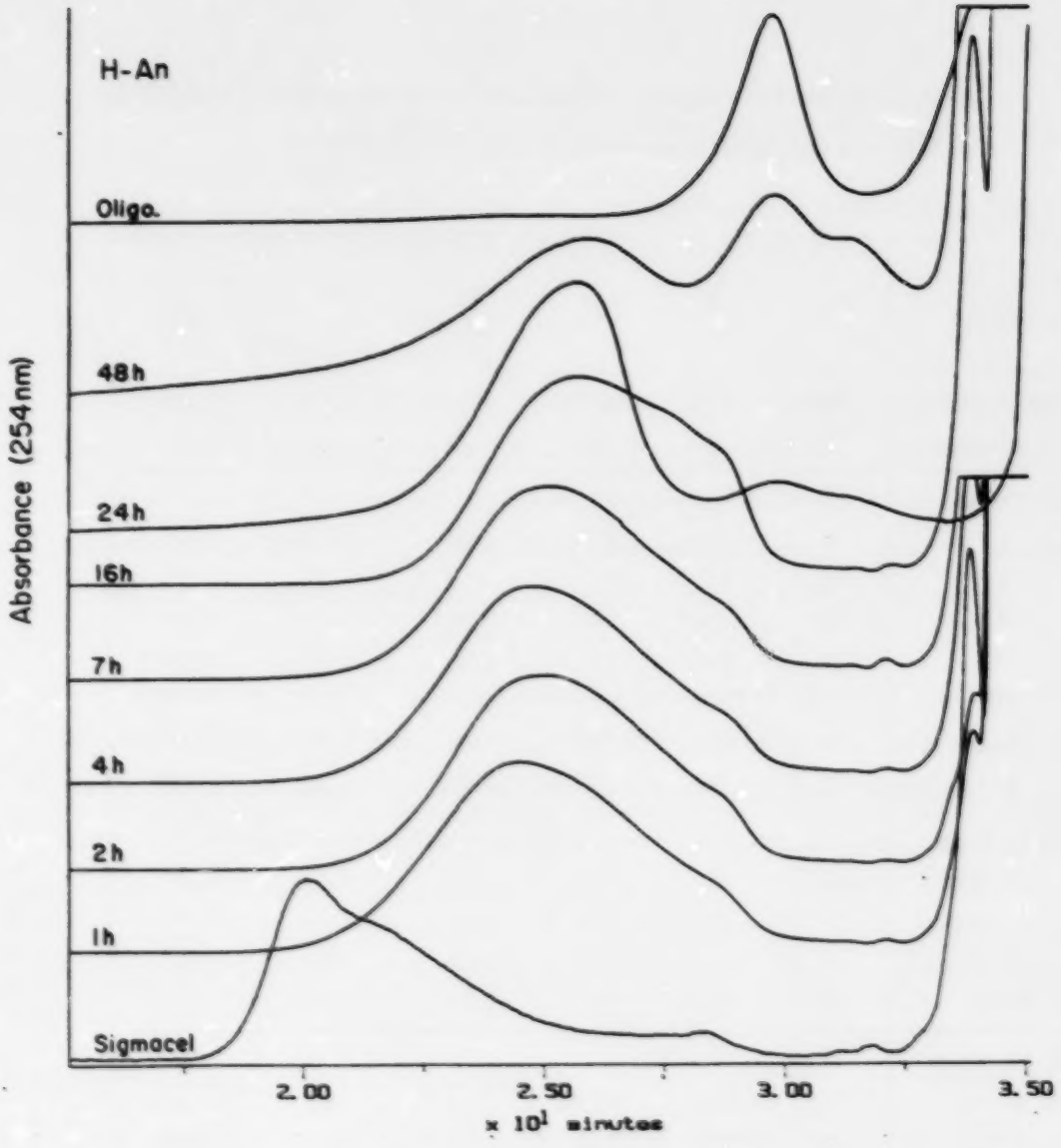

Figure 5.3: Size exclusion chromatography of cellulosic residues of H-An series

The evolution of the molecular weight distribution can be described by three steps:

- a first step which coincides with the rapid passage from the exponential distribution of the initial cellulose to a logarithmic distribution (CPC = 0.98) as the amorphous regions

Table 5.2: Average molecular weights, polydispersity and combined polydispersity coeffficient of cellulosic residues obtained by acid hydrolysis

Sample	$\overline{DP}_n$	$\overline{DP}_w$	$\overline{DP}_z$	$\frac{\overline{DP}_w}{\overline{DP}_n}$	$\frac{\overline{DP}_z}{\overline{DP}_w}$	CPC
Sigmacel	156	1218	2642	7.81	2.17	3.59
Oligosacch.	7	9	10	1.29	1.11	1.16
H-An Series						
1 h	50	172	602	3.44	3.50	0.98
2 h	45	141	452	3.13	3.21	0.98
4 h	43	124	349	2.88	2.81	1.02
7 h	42	116	324	2.76	2.79	0.99
16 h	32	77	175	2.41	2.27	1.06
24 h	35	78	152	2.23	1.95	1.14
48 h	15	66	156	4.40	2.36	1.86
H-Di Series						
20 min	42	261	2349	6.21	9.00	0.69
30 min	51	169	793	3.31	4.69	0.71
50 min	46	127	549	2.76	4.32	0.64
70 min	38	104	281	2.74	2.70	1.01
H-Co Series						
1-200	50	150	874	3.00	5.83	0.51
1-220	49	105	212	2.14	2.02	1.06
1-230	45	81	169	1.80	2.09	0.86
1-240	3	21	127	7.00	6.05	1.04

are hydrolysed;
- a second step where the logarithmic form of the distribution is maintained while the dispersity decreases;
- formation of a bimodal distribution suggesting the formation of insoluble and degraded residues with a "carbanilated" molecular weight around 5000.

5.1.4 X-ray diffraction studies

Table 5.3 shows the crystallinity indexes in the 002 and 101 planes and the crystallite width for the cellulosic residues of the three series. The evolution of these parameters is similar for the cellulosic residues obtained from the three processes. From a crystallinity index of 78% and a crystallite width of 34 Å of the initial cellulose, both parameters increase to a maximum at the $LODP_v$. Continuation of the hydrolysis leads to a decrease of these parameters.

From Figure 5.4, showing the crystallinity index of the 002 plane as a function of the degree of polymerization, it can be seen that the acid hydrolysis using the continuous process (H-Co) results in the production of the highest crystallinity cellulose. The cellulose obtained by this process keeps its crystalline properties at lower degree of polymerization.

Table 5.3: Crystallinity index (CrI) and crystallite width of cellulosic residues from the three series of hydrolysis experiments

Sample	$\overline{DP}_w$	$CrI_{(002)}$	$CrI_{(101)}$	Crystallite width (Å)
Avicel	250	88.4	65.6	41.4
Sigmacel	1218	78.4	45	33.8
H-An series				
1 h	172	85.9	59.4	41.4
2 h	141	87.1	58.6	41.4
4 h	124	86.2	58.1	39.5
7 h	116	86	58.1	41.4
16 h	77	82.2	48.4	38.4
24 h	78	79	45.6	37.9
48 h	66	67.5	24.2	26.8
H-Di series				
20 min	261	88	64.5	40.5
30 "	169	89.0	67.7	41.6
50 "	127	85.7	58.1	44.1
70 "	104	80.3	46.4	40.9
H-Co series				
1-200	150	94.1	76.5	50.7
1-220	105	90.1	66.7	50.4
1-230	81	88.9	67.9	47.4
2-200	---	81.7	50	42.0
1-240	21	63.7	18.5	23.6
2-220	---	53.8	0	---
2-230	---	0	0	---

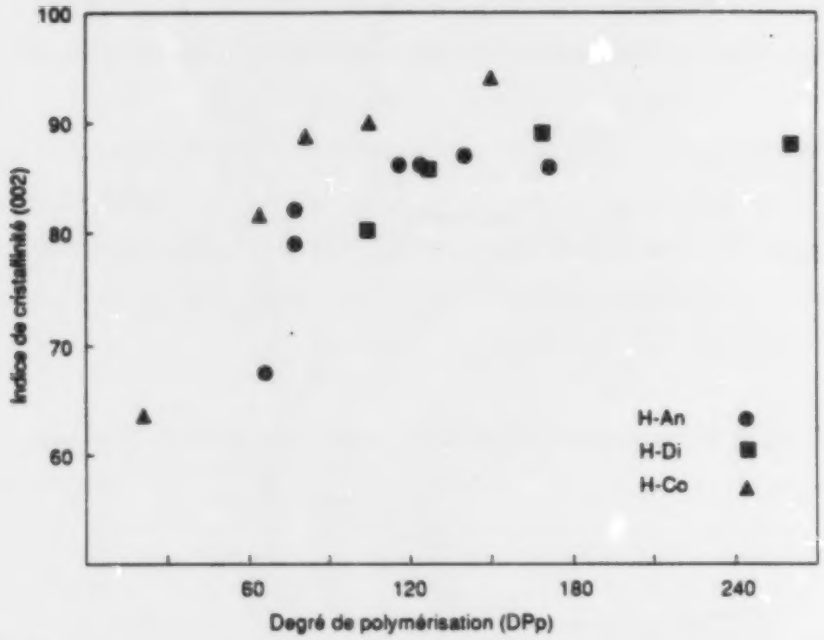

Figure 5.4: Crystallinity index as a function of weight average degree of polymerization ($\overline{DP}_w$) of cellulosic residues from the three series of experiments

5.1.5 ^{13}C CP/MAS solid state NMR

The CP/MAS NMR spectra have been acquired following the procedure described in Section 3.3.2. Figure 5.5 shows the NMR spectra of cellulosic residues of H-An series from 0 to 250 ppm of chemical shift. The 60-120 ppm area represents the different carbons of the cellulose. The study of this area indicates that a part of the cellulose remains undegraded even at the lowest DP. Furthermore, the shape of the dual peaks for carbon 4 (84 and 89.5 ppm) and carbon 6 (63 and 66 ppm) seems to indicate that the crystallinity of the cellulosic residues continuously increases as a function of the degree of polymerization.

As proposed by Hemmingson and Newman[35], we have calculated the ratio of the carbon 4 peaks which can be related to the crystallinity. The results are shown in Table 5.4 together with the crystallinity index obtained by X-ray diffraction and the glucose potential of the

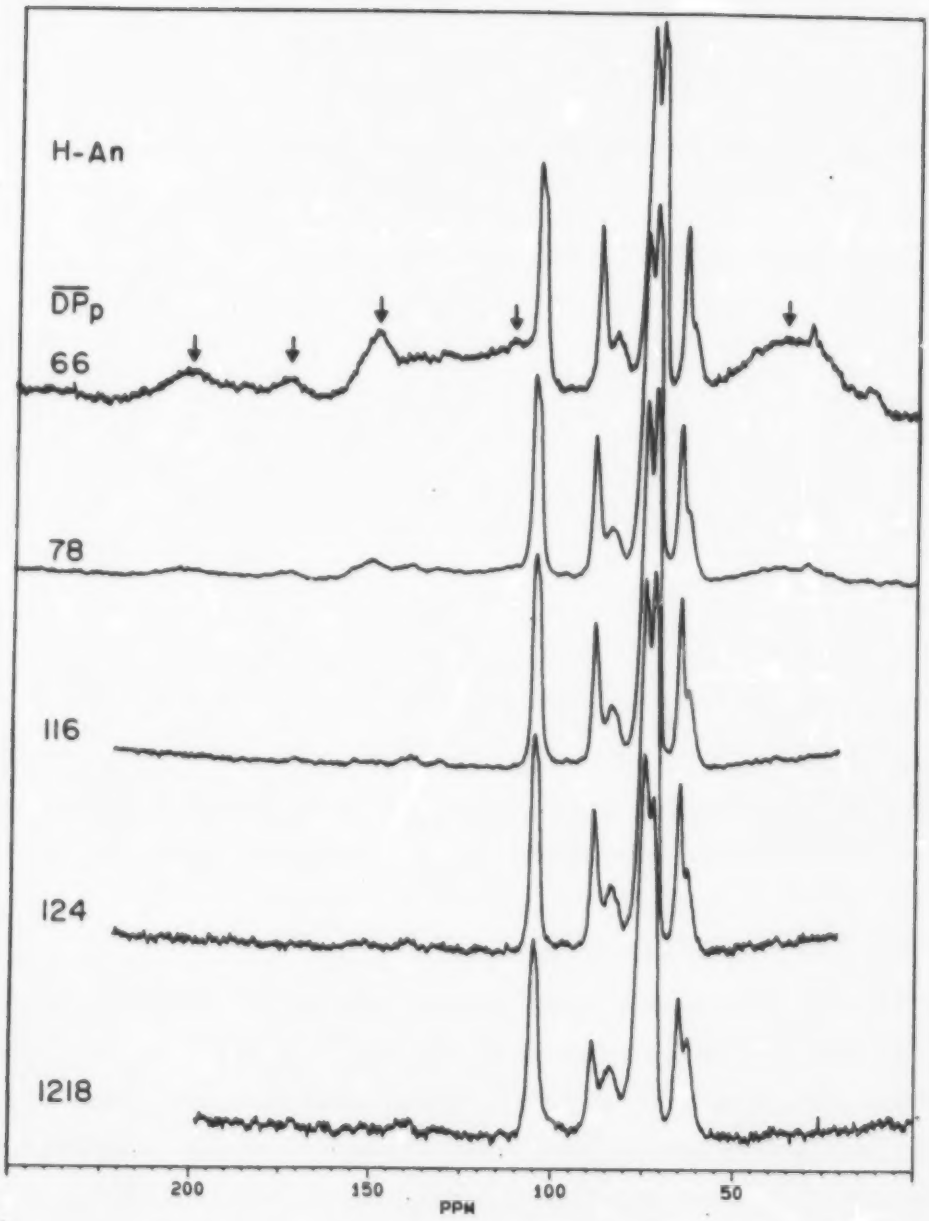

Figure 5.5: ^{13}C NMR (CP/MAS) spectra of cellulosic residues of H-An series (0-250 ppm)

residues. The crystallinity index decreases along with the glucose potential while the micro-order as evaluated by NMR increases continuously. The interpretation of these results are the following:

- both order indexes increase in the first stage of the hydrolysis as the amorphous regions are hydrolysed;
- once the crystallinity has reached a maximum due to the disappearance of the amorphous regions, hydrolysis and degradation of the crystallites occur simultaneously at their surfaces;

Table 5.4: Degree of order of cellulosic residues as determined by NMR and X-ray diffraction

Sample	C_{4C}/C_{4Am}	ICr_{002} (%)	Glucose potential Résidues (%)
Sigmacel	1.33	78	100
H-An series			
4 h	2.07	86	95
7 h	2.31	86	96
24 h	2.95	79	70
48 h	3.36	67	31
H-Co series			
1-230	1.95	89	63
1-240	2.60	64	19

- because the X-ray diffraction measures the concentration of the ordered region in the whole sample, the decrease of the crystallinity index is correlated with the degradation of the cellulosic residues;
- the NMR index is unaffected by degradation because it is related to the carbon 4 of the

intact glucose units. Consequently, this particular index should stay stable;

- the fact that this index increases is an indication that a gradient of crystallinity exits in the crystallites from the surface to the center.

The other region of the spectra of Figure 5.5 suggests the formation of new chemical structures following the acidic degradation of the cellulose. Four type of chemical structures can be related to the chemical shift of the peaks:

- carbonyl groups (ketone) at 210 ppm;
- carbonyl groups (carboxylic acid) at 175 ppm;
- insaturated carbons in the 110-160 ppm area with specific peaks at 110 and 150 ppm which can be related to a double bond formed between carbons 5 and 6;
- saturated aliphatic carbon in the 10-50 ppm area.

5.1.6 Discussion on a chemical model for dilute acid hydrolysis of cellulose

When cellulose is hydrolysed with dilute inorganic acid for a long period of time or at an elevated temperature, a chemical degradation of the cellulose occurs as the depolymerization and the solubilization proceed. This degradation begins at the LODP step but proceeds faster after this step. The principal effect of this degradation is a decrease of the glucose potential of the cellulosic residue.

The existing model for cellulose hydrolysis attributes the low glucose yield only to the reversion and degradation reactions of glucose in solution. In order to explain our results, we have to consider the degradation of the cellulose in the solid state as it is proposed in Figure 5.1.

The degradation must proceed via dehydration and oxidation reactions identical for all processes but activated by temperature. Formation of ketonic groups on carbons 2 and 3 and/or

carboxylic acids on carbons 2, 3 and 6 have been observed. Furthermore, dehydration reactions conducting to double bond formation on C_2-C_3 and C_3-C_6 have been demonstrated. Processes using high temperature lead to condensation reactions which are responsible for the formation of thermal stable structures probably of aromatic nature. Figure 5.6 shows the different structures induced by dilute acid hydrolysis processes.

The degradation results in the formation of an insoluble non-glycosidic residue representing 5% of the initial cellulose. This residue has a molecular weight around 3000.

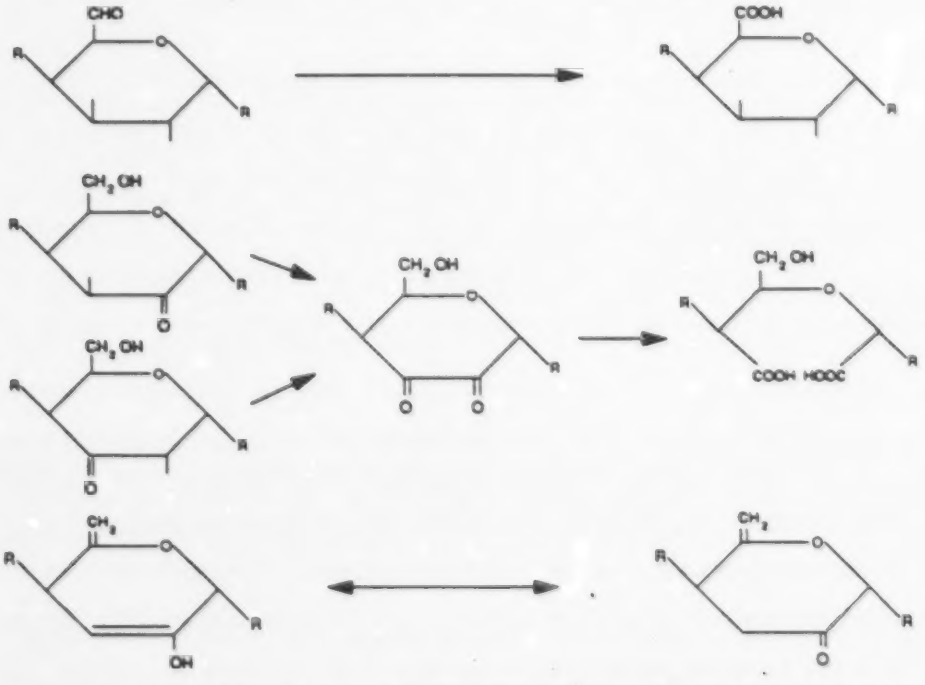

Figure 5.6: Possible structures of degraded cellulose following partial dilute acid hydrolysis

5.2 Model for depolymerization by ethylene glycol solvolysis processes

5.2.1 Introduction

Organosolvolytic treatment of wood to produce pulp has been largely investigated during the last decade[56]. In the meantime organosolv processes have been recognized as an important tool for pretreatment of lignocelluloses and separation of their constitutive components. It has been shown that pure ethylene glycol[57] or ethylene glycol/water system[58,59] is perhaps the most promising candidate among polyhydroxy alcohols for organosolv-pulping.

We have recently demonstrated using a small continuous reaction unit that the selective removal of hemicellulose and lignin can be achieved by a direct solvolytic treatment using ethylene glycol[60]. We have also proven that the residual cellulose can be depolymerized down to very low degrees of polymerization when subjected to a second solvolytic treatment in ethylene glycol at high temperature[61]. Furthermore, the degree of polymerization of the cellulosic residues could be controlled, within certain limits, by the reaction severity (temperature and residence time) of the treatment[62].

The depolymerization and destructuration phenomena have been further studied in our laboratory using a commercial cellulose[63]. The results have confirmed the previous work of Vanasse et al. and moreover have shown that the depolymerization of the cellulose is the predominant mechanism down to the level-off degree of polymerization ($\overline{DP}_v$ = 130). Beyond this level, the depolymerization process leads to extensive solubilization of the cellulose. The insoluble residue shows a marked enhancement of the enzymatic hydrolysis yield and rates even if the crystallinity of the cellulose remains unaffected by the depolymerization. The interest of these results lies in the possibility of preparing celluloses with very low degree of polymerization

which could be used as substrates in grafting or copolymerization studies. The solvolytic process could also be thought of as a pretreatment for enhancement of the yields of enzymatic hydrolysis of cellulose.

The usual way to produce cellulose with low degree of polymerization is by chemical treatment, especially acid catalysed hydrolysis which yields microcrystalline cellulose with an average degree of polymerization of 250 (e.g. Avicell™). We have shown that when the objective is the cellulose depolymerization beyond the level-off DP, the acid hydrolytic processes will induce important structural and chemical modifications in the cellulose[64].

We have begun in 1987 a detailed characterization of cellulosic residues obtained by solvolytic depolymerization process, operated in continuous mode, using ethylene glycol as solvent. Motivation for this study was derived from the need to understand the nature of the residues obtained from solvolysis. Comparison between the chemical modifications induced on the cellulose during depolymerization by aqueous acid hydrolysis processes and those, if any, induced by solvolytic depolymerization processes is useful for the understanding of the mechanisms involved in the process of depolymerization. A combination of thermogravimetric analysis (TG) and diffuse reflectance infrared spectroscopy (DRIFT) has been used in order to characterize the physical and chemical transformation of the cellulose as a function of the extent of depolymerization and the results have been published recently[64].

In the present report, we will complete the analytical characterization and propose a model for the mechanism of depolymerization of cellulose by ethylene glycol solvolysis.

5.2.2 Solvolysis experimental conditions

Experiments on solvolytic depolymerization of cellulose were conducted using two different substrates: (i) CELL series: a prototype cellulose derived from solvolysis of a hardwood

(*Populus deltoides*, clone D-38, grown in Brockville, Ontario). The purity of cellulose is greater than 95% and the average viscosity degree of polymerization ($\overline{DP}_v$) is 1100. (ii) BOIS series: a prototype of steam-exploded hardwood containing 80% of cellulose.

The concept developped at the University of Sherbrooke consists of a continuous solvolysis/liquefaction unit combining a pretreatment recirculation section and a tubular reactor section, capable of treating up to 4 kg per hour of material (on a dry basis). In the pretreatment section, the cellulose or wood suspension (10% w/w) was heated to a temperature between 200 and 240°C. The suspension was then continuously recirculated through a homogenizing valve (about 10 passes) across which a high pressure gradient (15-34 MPa) was maintained. The solvolysis takes place in a pressurized (4-7 MPa) tubular reactor maintained at constant temperatures (320-350°C) where the suspension was treated for short residence times (4-9 min). Finally, the residual cellulose was washed with water, dried at 60°C and weighed.

The Table 5.5 shows the experimental conditions, conversions, $\overline{DP}_w$ and glucose potential of of cellulosic residues of both solvolytic series (CELL and BOIS). The most relevant result of Table 5.5 is that the glucose potential of the cellulosic residues is always greater than 95%, even under the most severe conditions.

Table 5.5: Experimental conditions, conversions, $\overline{DP}_v$ and glucose potential of cellulosic residues from both solvolytic series (CELL and BOIS)

Sample	Conditions		Conversion	Cellulosic residue		Lignin
	T(°C)	t (min)	(%)	$\overline{DP}_v$	Glucose potential (%)	(%)
CELL series						
Initial	---	---	---	1350	---	2-8
1	312	7	10	796	---	0.0
2	316	9	27	451	97.2	2.2
3	321	5	33	406	95.3	3.0
4	327	6	33	322	98.3	0.2
5	337	4	40	185	---	3.0
6	342	6	41	187	---	0
7	347	4	66	105	96.5	0
BOIS series						
Exploded wood	---	---	---	855	97.7	15.5
1	311	4	23	265	95.4	8.9
2	321	4	26	219	97.0	1.8
3	333	4	30	176	---	---
4	340	4	34	142	---	---
5	345	4	50	101	98.2	---
6	320	9	55	67	96.8	---
7-9	336-350	9	100	---	---	---

5.2.3 Molecular weight distribution of cellulosic residues obtained by solvolysis

The molecular weight distribution of cellulosic residues have been obtained by size exclusion chromatography of their carbanilate derivatives as described in Section 3.1.2. Figure 5.7 shows the chromatograms of exclusion of both series of cellulosic residues which are identified by their respective weight average molecular weight.

The most important point issued from the evolution of the molecular weight distribution is that the form of the distribution is modified very slowly as a function of the severity. The dispersity coefficient ($\overline{DP}_w/\overline{DP}_n$) decreases but never reaches the value of two. Furthermore, the distribution remains exponential. This indicates that there is no rapid hydrolysis of the amorphous region and stabilization of the degree of polymerization of the crystalline cellulose as in the case of acid hydrolysis. These observations are consistent with the fact that the solvolytic depolymerization is not restrained by accessibility criteria and can occur anywhere inside or at the surface of the microfibril, in the amorphous or crystalline regions.

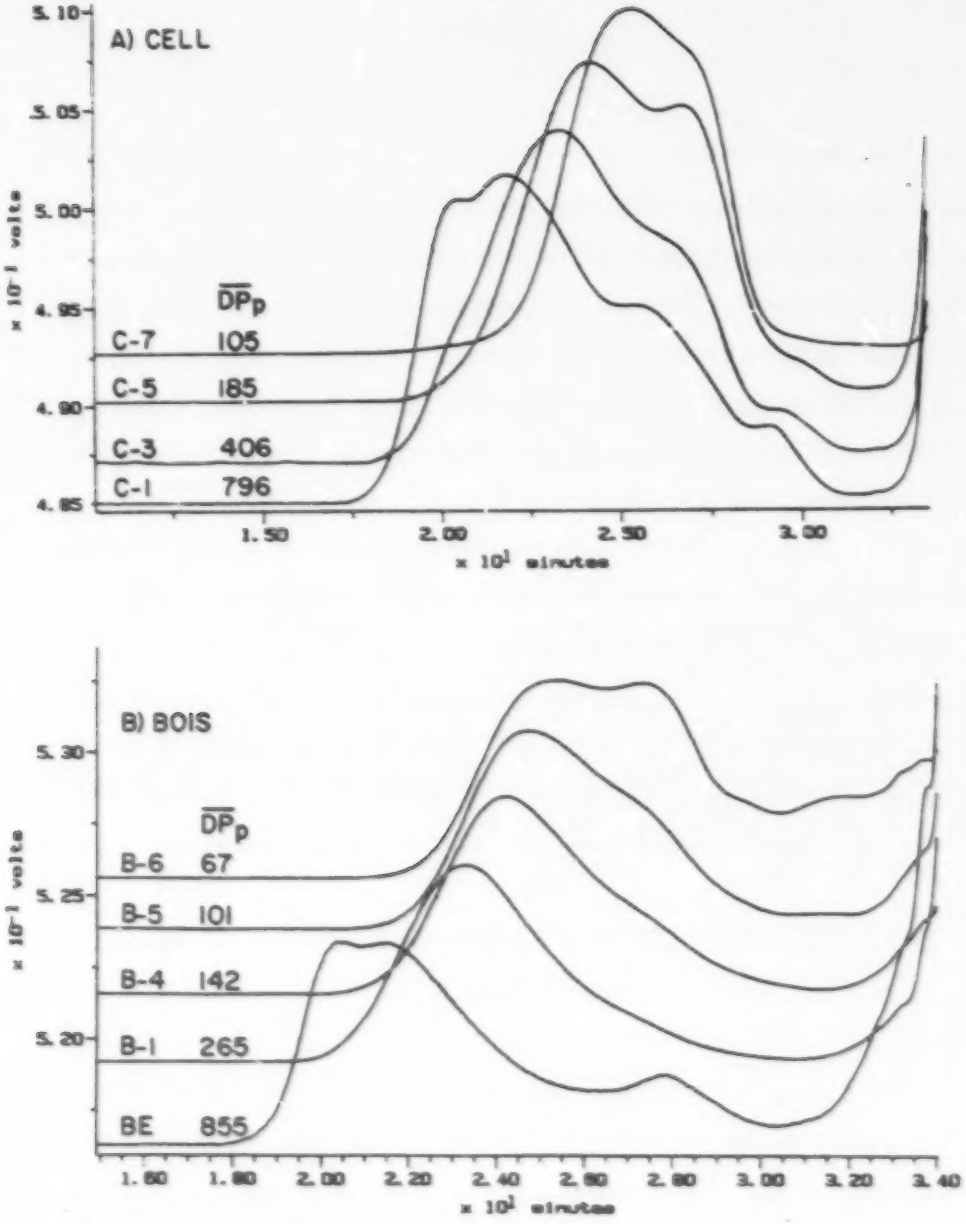

Figure 5.7: Size exclusion chromatography of cellulosic residues of A) CELL series and B) BOIS series

5.2.4 X-ray diffraction studies

The results obtained from X-ray diffraction analysis of the cellulosic residues from both series are presented in Table 5.6. These results confirm our interpretation of the evolution of the molecular weight distribution. The crystallinity index of the cellulosic residues increases slowly as the cellulose is depolymerized and not rapidly as in the acid hydrolysis case. The explanation for this behavior can be the persistence of an amorphous region while depolymerization of crystalline regions occurs. A completely random depolymerization not affected by accessibility criteria can explain this phenomenon.

Table 5.6: Crystallinity index and crystallite width of cellulosic residues of CELL and BOIS

Sample	$\overline{DP}_w$	$CrI_{(002)}$	$CrI_{(101)}$	Crystallite width (Å)
Avicel	250	88.4	65.6	41.4
Sigmacel	1218	78.4	45	33.8
CELL series				
1	796	78.5	48.7	34.9
3	406	81.4	57.5	38.4
5	185	83.7	60.5	39.5
6	187	83	60	39.5
7	105	88.5	69.4	51.0
BOIS series				
Initial wood	---	62.3	21.6	20.5
Exploded wood	855	80.4	54.2	41.4
1	265	87.5	67.6	43.1
4	142	89.7	71.4	49.4
5	101	87.5	68.4	45.3
6	67	83.7	61.5	44.1

5.2.5 ^{13}C CP/MAS solid state NMR

As presented in the previous report[7] and published recently[65], no chemical modification of the cellulosic residues has been detected even for the residues obtained from the more intense treatment. For a confirmation of this observation, the CP/MAS NMR spectra have been acquired following the procedure described in Section 3.3.2. Figure 5.8 shows characteristic NMR spectra of cellulosic residues obtained by ethylene glycol solvolysis from 0 to 250 ppm of chemical shift. As it can be observed from Figure 5.8, no chemical modification of the cellulose can be detected. Furthermore, the index of order, as measured from the C-4 doublet (80-90 ppm), corresponds perfectly to the crystallinity index measured by X-ray diffraction.

We have also determined the T_2 relaxation times of the cellulosic residues which represent the spin-spin relaxation. These have been determined by the spin echoes experiment. The results are presented in Table 5.7 and suggest the presence of two relaxation phenomena respectively described by a short T_2 of 0.54 ± 0.12 ms and a longer one of 1 ms which increases around 2 ms for the two residues.

A longer T_2 is attributed to the relaxation of domains which are more mobile. The ratio T_2 long / T_2 short for CELL-3 and BOIS-6 are greater than 3.5 and allow us to conduct the Goldman-Shen experiment[66]. This experiment allows the observation of the magnetization of the mobile domain only while the magnetization of the rigid domain has been almost completely relaxed. If this observation is made as a function of time, we are then able to observe the transfer of magnetization from the mobile to the rigid domain[67]. Knowing the diffusion speed, the dimension of the mobil domain can be calculated from this experiment[68]. Table 5.8 shows the dimension of the mobile domains of these cellulosic residues calculated with the equations accounting for uni-, bi- and tridimensional domains.

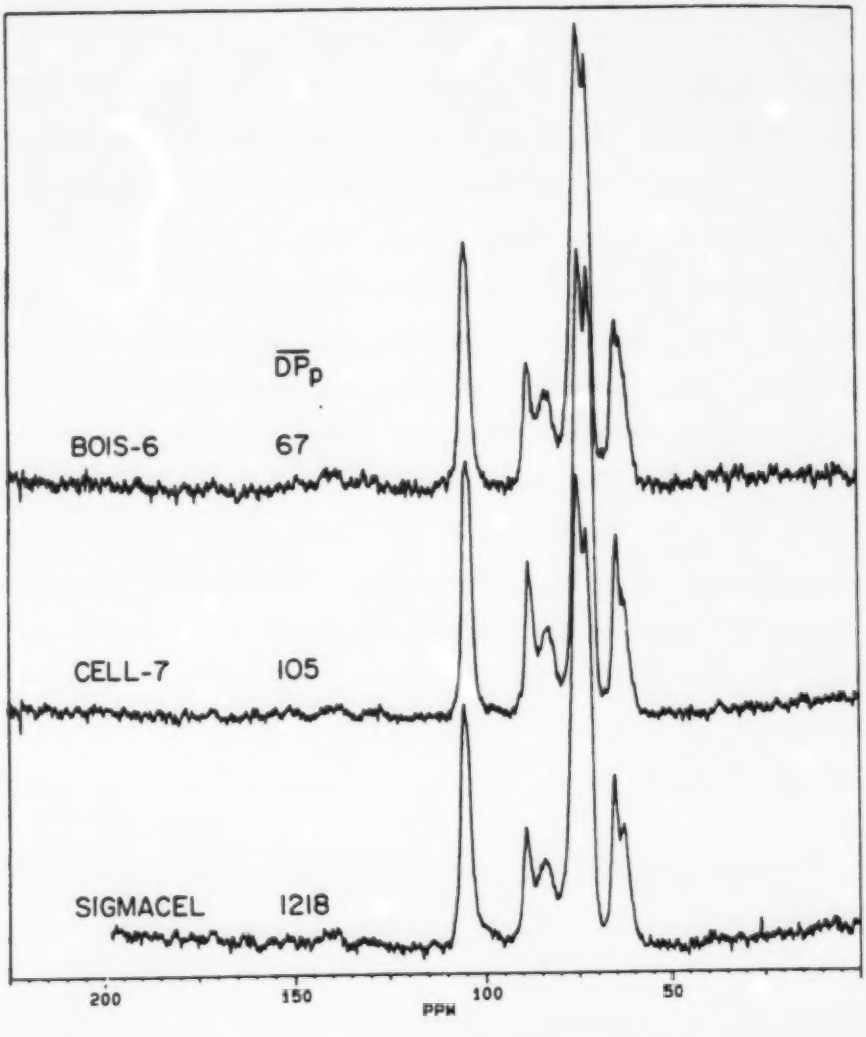

Figure 5.8: ^{13}C solid state NMR spectra of Sigmacel and residues CELL-7 and BOIS-6 (0-225 ppm)

Table 5.7: Determination of T_2 (ms) relaxation time

Sample	T_2 (short)	T_2 (long)
Sigmacel	0.38	0.69
CELL series		
3	0.53	2.36
6	0.51	1.07
7	0.50	1.07
BOIS series		
1	0.38	---
4	0.68	1.13
5	0.44	1.12
6	0.71	1.87

Table 5.8: Mobil domain dimension as a function of the dimensionality

Dimensionality	CELL-3 $\overline{b}$ (Å)	BOIS-6 $\overline{b}$ (Å)
1 D	15.8	18.7
2 D	22.9	25.7
3 D	28.5	31.2

The general conclusion from these results is that the depolymerization of the cellulose by ethylene glycol solvolysis conducts to the formation of regions where the glucose units are more mobile but surrounded by rigid areas. This can be related to the depolymerization occuring in the crystalline region of the cellulose and can be represented by the concept illustrated in Figure 5.9.

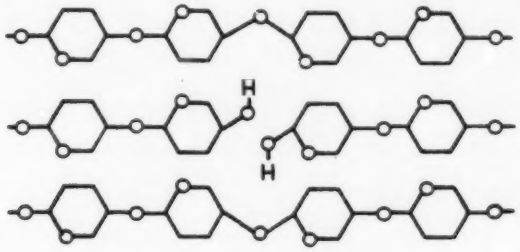

Figure 5.9: Mobil domain resulting of glycosidic bond breaking into a rigid structure

5.2.6 Model for depolymerization of cellulose by solvolysis in ethylene glycol

The results presented in the Section 5.2 allow us to propose a physical model for the depolymerization of the cellulose by ethylene glycol solvolysis which is illustrated in Figure 5.10. We will discuss about the chemical mechanism later but we will for now assume that the high temperature treatment conducts to a random mechanism of depolymerization and that the creation of chain ends does not induce unzipping reactions like the pyrolysis mechanism.

From the initial cellulose (A) in Figure 5.10 showing an amorphous region surrounded by two crystalline regions, the solvolytic treatment yields the structures B, C or D as a function of the severity. At the beginning, the depolymerization gives a cellulose which is fibrillar (Step B) and has kept its hydrogen bonding network. The broken bonds are responsible for the mobile

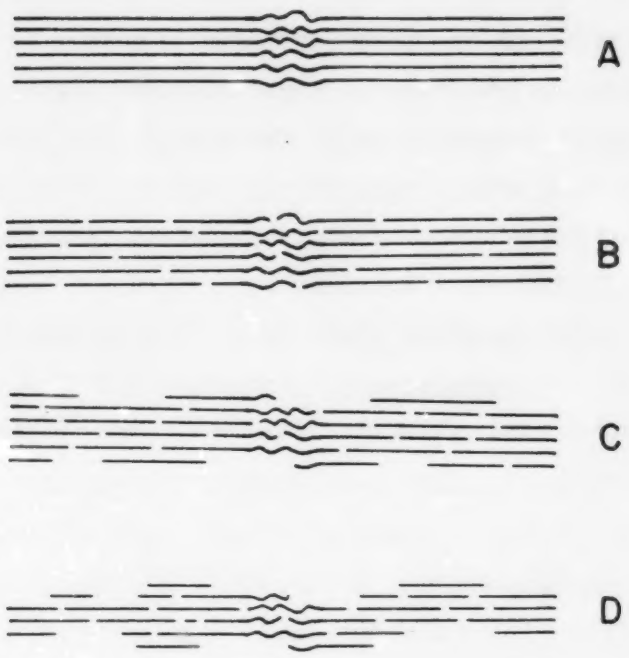

Figure 5.10: Model for the depolymerization of cellulose by ethylene glycol solvolysis

domain formed in the microfibril. When the chains at the surface become shorter (oligosaccharides), they go into solution (Step C) where they are rapidly depolymerized and the glucose is degraded[69]. The remaining microfibril is more depolymerized and a larger amount of short chains are solubilized (Step D) but the cohesion and the allomorphic structure remain.

When treating the cellulose at temperatures higher than 300°C in a hydroxylated solvent, the mechanism of depolymerization can be either pyrolytic or hydrolytic in nature. But both of these mechanisms induced degradation of the cellulosic residue in normal conditions. Consequently we can state that the ethylene glycol plays a protective role assuring the production of undegraded depolymerized cellulose. We think that this role implies a complete penetration

of the ethylene glycol in the cellulosic structure as detected by swelling. In the case of a hydrolytic mechanism, the role of the ethylene glycol involves the inhibition of the degradation reactions probably by hydrogen bonding with the cellulose. In the case of a pyrolytic mechanism, the role of the ethylene glycol should be chemically more profound. The pyrolysis involves the formation of very reactive radicals which induce degradation and unzipping depolymerization of the cellulose. The inhibition of degradation implies a control of the radical reaction. It is possible that ethylene glycol establishes covalent bonds with the new reducing ends formed by depolymerization. However, no evidence for this mechanism has been observed and more studies are needed for the complete understanding of the depolymerization mechanism.

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